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Stefan Offermanns
Walter Rosenthal
Editors

Encyclopedia of Molecular Pharmacology

2nd Edition

 Springer

Offermanns · Rosenthal
Eds.



Encyclopedia of
Molecular Pharmacology

2nd Ed.

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STEFAN OFFERMANN AND WALTER ROSENTHAL (Eds.)

Encyclopedia of Molecular Pharmacology (2nd edition)

With 487 Figures* and 171 Tables



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Preface to the First Edition

The era of pharmacology, the science concerned with the understanding of drug action, began only about 150 years ago when Rudolf Buchheim established the first pharmacological laboratory in Dorpat (now, Tartu, Estonia). Since then, pharmacology has always been a lively discipline with “open borders”, reaching out not only to other life sciences such as physiology, biochemistry, cell biology and clinical medicine, but also to chemistry and physics. In a rather successful initial phase, pharmacologists devoted their time to describing drug actions either at the single organ level or on an entire organism. Over the last few decades, however, research has focused on the molecular mechanisms by which drugs exert their effects. Here, cultured cells or even cell-free systems have served as models. As a consequence, our knowledge of the molecular basis of drug actions has increased enormously. The aim of *Encyclopedic Reference of Molecular Pharmacology* is to cover this rapidly developing field.

The reductionist approach described above has made it increasingly important to relate the molecular processes underlying drug actions to the drug effect on the level of an organ or whole organism. Only this integrated view will allow the full understanding and prediction of drug actions, and enable a rational approach to drug development. On the molecular or even atomic level, new disciplines such as bioinformatics and structural biology have evolved. They have gained major importance within the field but are particularly relevant for the rational development and design of new drugs. Finally, the availability of the complete genome sequence of an increasing number of species provides a basis for systematic, genome-wide pharmacological research aimed at the identification of new drug targets and individualised drug treatment (pharmacogenomics and pharmacogenetics). All these aspects are considered in this encyclopedia.

The main goal of the *Encyclopedia* is to provide up-to-date information on the molecular mechanisms of drug action. Leading experts in the field have provided 159 essays, which form the core structure of this publication.

Most of the essays describe groups of drugs and drug targets, with the emphasis not only on already exploited drug targets, but also on potential drug targets as well. Several essays deal with the more general principles of pharmacology, such as drug tolerance, drug addiction or drug metabolism. Others portray important cellular processes or pathological situations and describe how they can be influenced by drugs. The essays are complemented by more than 1600 keywords, for which links are provided. By looking up the keywords or titles of essays highlighted in each essay, the reader can obtain further information on the subject. The alphabetical order of entries makes the *Encyclopedia* very easy to use and helps the reader to search successfully. In addition, the names of authors are listed alphabetically, together with the title of their essay, to allow a search by author name.

Apart from very few exceptions, the entries in the main text do not contain drug names in their titles. Instead, drugs that are commonly used all over the world are listed in the Appendix. Also included in the Appendix are four extensive sections that contain tables listing proteins such as receptors, transporters or ion channels, which are of particular interest as drug targets or modulators of drug action.

The *Encyclopedia* provides valuable information for readers with different expectations and backgrounds (from scientists, students and lecturers to informed lay-people) and fills the gap between pharmacology textbooks and specialized reviews.

All the contributing authors as well as the editors have taken great care to provide up-to-date information. However, inconsistencies or errors may remain, for which we assume full responsibility. We welcome comments, suggestions or corrections and look forward to a stimulating dialog with the readers of the *Encyclopedic Reference of Molecular Pharmacology* whether their comments concern the content of an individual entry or the entire concept.

We are indebted to our colleagues for their excellent contributions. It has been a great experience, both personally and scientifically, to interact with and learn from the 200 plus contributing authors. We would also like to thank Ms. Hana Deuchert and Ms. Katharina Schmalfeld for their excellent and invaluable secretarial assistance during all the stages of this project. Within Springer-Verlag, we are grateful to Dr. Thomas Mager for suggesting the project and to Frank Krabbes for his technical expertise. Finally, we would like to express our gratitude to Dr. Claudia Lange for successfully managing the project and for her encouraging support. It has been a pleasure to work with her.

Heidelberg/Berlin, June 2003

STEFAN OFFERMANN AND WALTER ROSENTHAL

Preface to the Second Edition

The first edition of the *Encyclopedic Reference of Molecular Pharmacology* was well received by its readers, thanks to the excellent work done by the authors, of whom most have contributed to the second edition as well. The basic structure of the Encyclopedia has remained unchanged. It is primarily based on essays, which have been updated, and their number has been increased to 225 to include many new exciting areas. These essays cover important drugs and drug targets, but also general principles of pharmacology as well as cellular processes and pathological situations which are relevant for drug action. In addition, there are about xy key words linked to the essays. The Encyclopedia is complemented by an Appendix, which has been greatly enlarged, listing more than 700 drugs and more than 4,000 proteins that act as receptors, membrane transport proteins, transcription factors, enzymes or adhesion molecules.

During the preparation, we greatly enjoyed the interaction with all our colleagues who contributed to this reference work. It has been a pleasure and an enriching experience to deal with so many facets of pharmacology. We are very thankful to the contributing authors for the careful updating of their essays, and, in particular, we would like to express our gratitude to the more than xy new authors who have written excellent essays on novel topics. Finally, we would like to thank Dr. Michaela Bilic and Simone Giesler from Springer for their enthusiasm throughout the project and their constant support.

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2DE

- Two-dimensional Electrophoresis
- Proteomics

14-3-3 σ

The 14-3-3 proteins constitute a family of abundant, highly conserved and broadly expressed acidic polypeptides. One member of this family, the 14-3-3 σ isoform {sigma}, is expressed only in epithelial cells and is frequently down-regulated in a variety of human cancers and plays a role in the cellular response to DNA damage. The 14-3-3s generally form heterodimers with other family members, but 14-3-3 σ preferentially forms homodimers in cells. Three amino acids that are completely conserved in all other 14-3-3s, are not present in 14-3-3 σ . These amino acids unique to 14-3-3 σ confer a second ligand-binding site involved in 14-3-3 σ -specific ligand discrimination.

- 14-3-3 Proteins

A Kinase Anchoring Proteins (AKAPs)

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Synonyms

Protein kinase A anchoring proteins

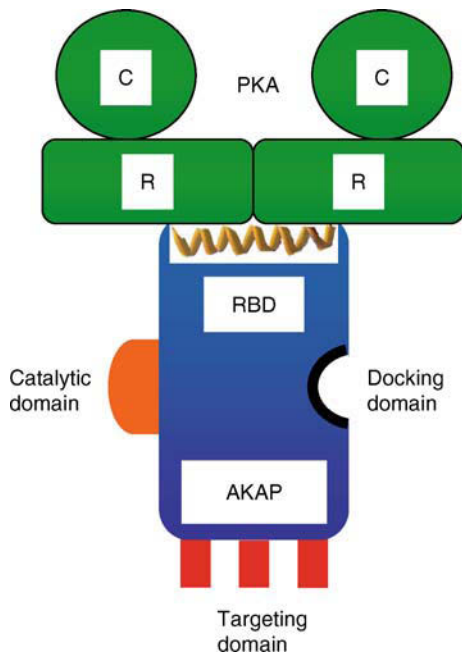
Definition

AKAPs are a diverse family of about 75 scaffolding proteins. They are defined by the presence of a structurally conserved protein kinase A (PKA)-binding domain. AKAPs tether PKA and other signalling proteins to cellular compartments and thereby limit and integrate cellular signalling processes at specific sites. This compartmentalization of signalling by AKAPs contributes to the specificity of a cellular response to a given external stimulus (e.g. a particular hormone or neurotransmitter).

Basic Mechanisms

AKAP-dependent Control of cAMP/PKA Signalling

A large variety of extracellular stimuli including hormones and neurotransmitters elicit the generation of the second messenger cyclic adenosine monophosphate (cAMP). Cyclic AMP binds to several effector proteins including ion channels, cAMP-dependent guanine-nucleotide-exchange factors (Epacs) and PKA. The latter is the main effector of cAMP. Binding of four molecules of cAMP activates the kinase. Activated PKA transfers a phosphate group from adenosine triphosphate (ATP) to consensus sites on many different substrate proteins and thereby modulates their activity. It appears that different external stimuli mediate activation of specific pools of PKA located at defined sites within cells (compartments) including, for example mitochondria, nuclei, exocytic vesicles, sarcoplasmic reticulum and the cytosol [1]. A kinase anchoring proteins (AKAPs; Fig. 1) tether PKA to such cellular compartments and allow for its local activation, and consequent phosphorylation of particular substrates in close proximity [2]. Spatial and temporal coordination of PKA signalling through compartmentalization by AKAPs is considered essential for the specificity of PKA-dependent cellular responses to a particular external stimulus [3, 4]. AKAP–PKA interactions play a role in a variety of cellular processes including β -adrenoceptor-dependent regulation of cardiac myocyte contraction (Fig. 2), vasopressin-mediated water reabsorption, proton secretion from gastric parietal cells, modulation of insulin secretion from pancreatic β cells and T cell receptor signalling. A typical AKAP is AKAP18 α , also termed AKAP15. It tethers PKA to ►L-type Ca²⁺ channels in

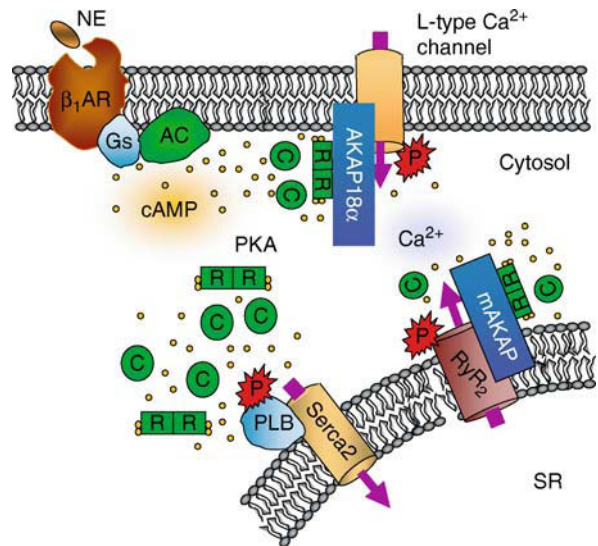


A Kinase Anchoring Proteins (AKAPs).

Figure 1 Model of an A kinase anchoring protein (AKAP). The unifying characteristic of AKAPs is the presence of a structurally conserved binding domain for the dimer of regulatory (R) subunits of PKA (RBD, regulatory subunit binding domain). In the inactive state, PKA forms a tetramer consisting of a dimer of R subunits each bound to one catalytic subunit (C). Binding of two molecules of cAMP to each R subunit causes a conformational change and release of the C subunits, which in the free form phosphorylate substrate proteins in close proximity. The RBD in all AKAPs with pericentrin as the only exception forms an amphipathic helix that docks into a hydrophobic pocket formed by the dimerization and docking domain of R subunits. The targeting domain, which tethers the AKAP complex to cellular compartments and docking domains, which bind further signalling proteins (e.g. phosphodiesterases, phosphatases or other kinases) are specific for individual AKAPs. A few AKAPs possess catalytic activity such as the RhoGEF-activity in AKAP-Lbc conferred by a DH domain. The proteins within the AKAP family are without obvious sequence homology.

cardiac myocytes and skeletal muscle cells and facilitates their phosphorylation in response to β -adrenoceptor activation. The phosphorylation increases the open probability of the channel.

The tethering of PKA through AKAPs by itself is not sufficient to compartmentalize and control a cAMP/PKA-dependent pathway. Cyclic AMP readily diffuses throughout the cell. Therefore, discrete cAMP/PKA signalling compartments are only conceivable if this diffusion is limited. ► **Phosphodiesterases** (PDE) establish gradients of cAMP by local hydrolysis of the



A Kinase Anchoring Proteins (AKAPs).

Figure 2 β -adrenoceptor-induced increases in cardiac myocyte contractility depend on AKAP–PKA interactions. Stimulation of β -adrenoceptors (β_1 AR) on the surface of cardiac myocytes by binding of adrenergic agonists such as norepinephrine (NE), epinephrine or isoproterenol increases contractility of the heart. Agonist binding to the receptors activates the G protein G_s and adenylyl cyclase (AC), and consequent synthesis of cAMP which binds to regulatory (R) subunits of protein kinase A (PKA) inducing dissociation of catalytic (C) subunits (see also Fig. 1). The C subunits phosphorylate L-type Ca^{2+} channels located in the plasma membrane (plasmalemma) and ryanodine receptors (RyR_2) embedded in the membrane of the sarcoplasmic reticulum (SR). Phosphorylation of the two channel proteins increases their open probability and leads to an increase in cytosolic Ca^{2+} causing increased contractility. For the relaxation of cardiac myocytes, Ca^{2+} has to be removed from the cytosol. A pivotal role in this plays sarcoplasmic Ca^{2+} ATPase (SERCA). It pumps Ca^{2+} back into the SR. SERCA is inhibited when bound to phospholamban (PLB) and activated upon dissociation from PLB, which is induced by β -adrenoceptor-mediated PKA phosphorylation. Collectively, the PKA phosphorylation events increase cardiac myocyte contractility. The efficient phosphorylation of L-type Ca^{2+} channels occurs only if PKA is anchored to the channel by AKAP18 α . For the phosphorylation of RyR , PKA anchoring to this channel by mAKAP is a prerequisite. Further AKAPs are likely to be involved in PKA-dependent phosphorylation events in response to β -adrenoceptor stimulation (e.g. PLB).

second messenger and thereby regulate PKA activity locally. Several AKAPs interact with PDEs and thus play a role at this level of control. For example, the interaction of muscle-specific mAKAP with cAMP-specific PDE4D3 and the ► **ryanodine receptor** (RyR)

facilitates hydrolysis of cAMP in the vicinity of RyR at the sarcoplasmic reticulum of cardiac myocytes. Local cAMP hydrolysis keeps mAKAP-associated PKA activity low. An increase in the cAMP level exceeding the PDE4D3 hydrolyzing capacity activates PKA, which phosphorylates RyR and increases the open probability of this Ca^{2+} channel. PKA also phosphorylates mAKAP-bound PDE4D3 and thereby enhances PDE4D3 activity. This again increases local cAMP hydrolysis, switches off PKA, and eventually reduces RyR phosphorylation. This negative feedback loop regulating RyR phosphorylation is completed by association of mAKAP with protein phosphatase 2A (PP2A), dephosphorylating RyR. Dephosphorylation decreases the channel open probability of RyR.

AKAP-dependent Integration of Cellular Signalling

In addition to PKA, PDEs and protein phosphatases involved in cAMP signalling, AKAPs interact with other signalling proteins whose activation depends on second messengers other than cAMP, e.g. Ca^{2+} . AKAPs may bind additional kinases such as protein kinases C (PKC) and D (PKD), and further protein phosphatases such as calcium/calmodulin-dependent phosphatase (calcineurin, protein phosphatase 2B, PP2B). This scaffolding function allows AKAPs to integrate cellular signalling processes. For example, rat AKAP150 and its human ortholog AKAP79 bind PKA, PKC and calcineurin. In neurons, AKAP150-bound PKC is activated through a M_1 muscarinic receptor-induced pathway that depends on the G protein G_q and leads to elevation of cytosolic Ca^{2+} and diacylglycerol. AKAP150 interacts directly with M channels (K^+ channel negatively regulating neuronal excitability) and facilitates PKC phosphorylation and thereby inhibition of this channel. AKAP79 coordinates the phosphorylation of **▶AMPA channels**. Cyclic AMP-activated AKAP79-bound PKA phosphorylates and thereby activates the channels. A raise of cytosolic Ca^{2+} activates AKAP79-bound calcineurin, which in turn dephosphorylates the channels. The dephosphorylation mediates the rundown of AMPA channel currents.

AKAP-Lbc binds PKA, PKC and PKD and possesses intrinsic catalytic activity (Rho guanine nucleotide exchange factor (RhoGEF) activity). Through its RhoGEF activity it catalyses the exchange of GDP for GTP on the **▶small GTPase Rho**. The GTP form of Rho is active and induces the formation of F-actin-containing stress fibres. Agonists stimulating receptors coupled to the G protein G_s may mediate activation of AKAP-Lbc-bound PKA, which in turn phosphorylates AKAP-Lbc. Subsequently, a protein of the **▶14-3-3** family binds to the phosphorylated site and inhibits the RhoGEF activity. In contrast, agonists stimulating receptors coupled to the G protein G_{12} increase the RhoGEF activity.

AKAPs Optimise the Limited Repertoire of Cellular Signalling Proteins

Intriguingly, the same AKAP may coordinate regulation of different target proteins. In hippocampal neurons, AKAP150 positions PKA and calcineurin to modulate AMPA channels and maintains PKC inactive. In superior ganglial neurons, AKAP150 facilitates PKC phosphorylation of M channels while keeping PKA and calcineurin inactive. The difference is due to the interaction of AKAP150 with the scaffolding protein SAP97, which occurs in hippocampal neurons but not in superior ganglial neurons. SAP97 positions AKAP150 such that PKA and calcineurin are in close proximity to AMPA channels. Thus by variation of a single interacting partner an AKAP optimises the usage of the limited set of cellular signalling proteins.

In summary, the function of AKAPs goes far beyond controlling cAMP/PKA signalling by simply tethering PKA to cellular compartments and confining the access of PKA to a limited set of local substrates. AKAPs are scaffolds forming multiprotein signal transduction modules, recently termed “AKAPosomes” that coordinate and integrate cellular signalling processes.

Pharmacological Intervention

Disturbances of compartmentalized cAMP signalling in processes such as the ones mentioned above cause or are associated with major diseases including congestive heart failure, diabetes insipidus, diabetes mellitus, obesity, diseases of the immune system (e.g. AIDS), cancer and neurological disorders including schizophrenia. However, AKAPs participating in compartmentalized cAMP signalling networks are not targeted by drugs which are currently applied for the treatment of such diseases.

Recently, clinically relevant intracellular protein–protein interactions have gained much interest as potential drug targets. The cell-type specificity of such interactions and the finding that mostly only selected isoforms of proteins interact with each other offers great opportunities for highly selective pharmacological intervention. For targeting AKAP-dependent protein–protein interactions, peptides non-selectively displacing PKA from all AKAPs have been developed so far. For example, the peptides functionally uncouple PKA from L-type Ca^{2+} channels in cardiac myocytes by disruption of the AKAP18 α –PKA interaction that facilitates L-type Ca^{2+} channel phosphorylation (see above). This prevents β -adrenoceptor-induced increases in cytosolic Ca^{2+} , an effect resembling that of β -blockers. In renal collecting duct principal cells, vasopressin regulates water reabsorption from primary urine by triggering the PKA phosphorylation and subsequent redistribution of aquaporin-2 (AQP2) from intracellular vesicles into the plasma membrane. The redistribution depends on

the compartmentalization of PKA by AKAPs, one of which is AKAP18 δ . The PKA anchoring disruptor peptides displace PKA from AQP2-bearing vesicles and inhibit vasopressin-mediated water reabsorption, i.e. have an aquaretic effect. These examples suggest that cell-type specific pharmacological intervention at selected AKAP–PKA interactions is a feasible concept for the treatment of human diseases (e.g. cardiovascular disease or diseases associated with water retention).

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ABC-proteins

► ABC Transporters

ABC Transporters

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Synonyms

ATP-binding cassette proteins; ABC-proteins

Definition

The ►**ABC-transporter** superfamily represents a large group of transmembrane proteins. Members of this family are mainly involved in ATP-dependent transport processes across cellular membranes. These proteins are of special interest from a pharmacological point of

view because of their ability to transport numerous drugs, thereby modifying intracellular concentrations and hence effects.

Basic Characteristics

ATP-binding cassette (ABC-) proteins have been identified in all living organisms; they are present in plants, bacteria, and mammals. In humans the ABC-superfamily comprises about 50 members; on the basis of homology relationships this superfamily is organized in several subfamilies named ABCA to ABCF. Not all of them are pharmacologically important, for example, members of the A branch are mainly involved in lipid trafficking. ABCB2 as well as ABCB3, which are also termed as *transporter associated with antigen processing* (TAP), are involved in the transport of peptides presented by Class I HLA molecules. However, other ABC-transporters like ABCB1 (►**P-glycoprotein**, P-gp), ABCG2 (*breast cancer resistance protein*, BCRP) and several members of the C-branch are of high pharmacological relevance because they are involved in transport of several drugs; thereby affecting pharmacokinetic parameters.

ABCB1, for example, which is also known as P-glycoprotein (P-gp) and probably the best characterized ABC-transporter, has been identified as the underlying mechanism of a cancer-related phenomenon called ►**multidrug resistance** (therefore, P-gp is also termed *multidrug resistance protein* (MDR1)), which is characterized by the resistance of cancer cells against drug therapy. Interestingly, this phenomenon is not directed against a single drug or structurally related entities, but comprises unrelated compounds with different target structures. Meanwhile, besides P-gp further ABC-transporters have been identified to be involved in this process. For example, in 1992 Cole et al. identified the first member of the so-called *multidrug resistance related proteins* (MRP). The ►**MRP**-proteins (MRP1–MRP9) belong to the C-branch of the ABC-superfamily, which currently consists of a total of 13 members (ABCC1–ABCC13). In addition to ABCB1 and the ABCC family, a member of the ABCG family has recently been demonstrated to confer drug resistance. This protein called *breast cancer resistant protein* (►**BCRP**/ABCG2) was first identified in mitoxantrone resistant cell lines, which lack expression of P-gp or MRP1.

Topology and Structure

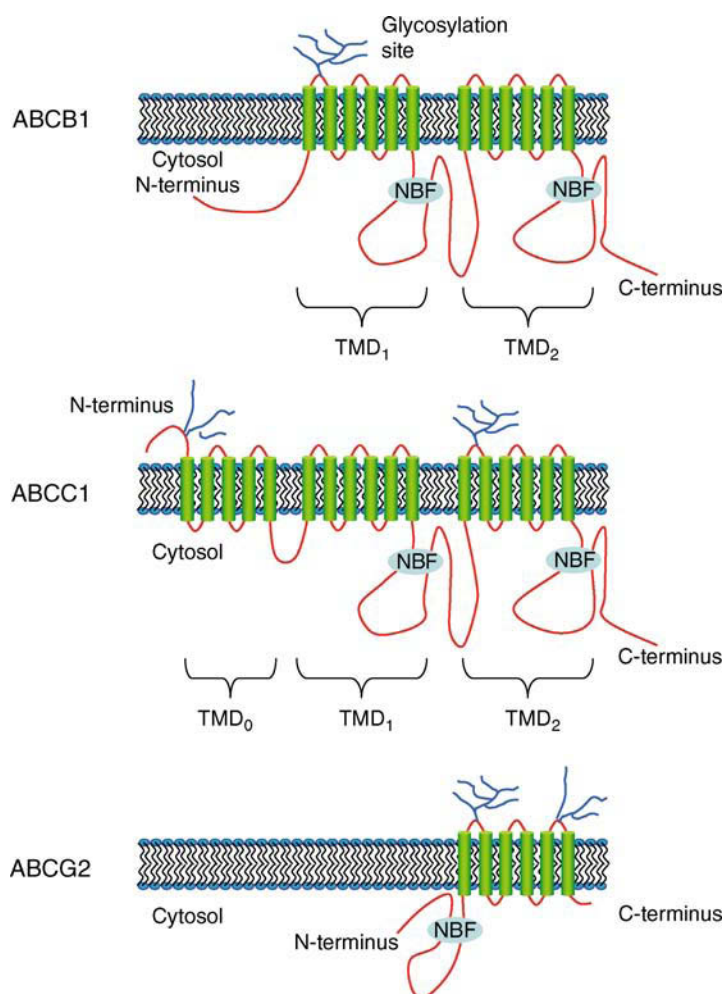
Most ABC-transporters, especially those located in the plasma membrane, are phosphorylated and glycosylated transmembrane proteins of different molecular weights (e.g., P-gp: 170 kDa; MRP2: 190 kDa; BCRP: 72 kDa). Topologically, most ABC-transporters show a similar structure: they are organized in two transmembrane domains (TMD), each consisting of six

α -helical, transmembranal segments and two ATP binding domains linked to the C-terminus of the TMDs. These domains, which are also termed *nucleotide binding folds* (NBFs), contain the highly conserved Walker A and B consensus motifs and the LSGGQ motif (also called C- or signature motif). While the Walker A and B motifs are also found in other ATP-hydrolyzing ATP proteins, the LSGGQ motif is unique for the ABC-transporters. The ATP hydrolysis catalyzed by the NBFs is a prerequisite for substrate binding and enables transport against a substrate gradient. In addition to these general characteristics, several members of the ABCC-family (e.g., ABCC1–3) contain a further N-terminal TMD, which, however, is not required for transport activity. In contrast to the other TMDs, this N-terminal TMD contains five transmembranal segments and lacks the NBF. Besides this structural variant some other ABC-transporters (e.g., ABCB2, 3 (TAB1 and 2), as well as ABCG2) contain only one

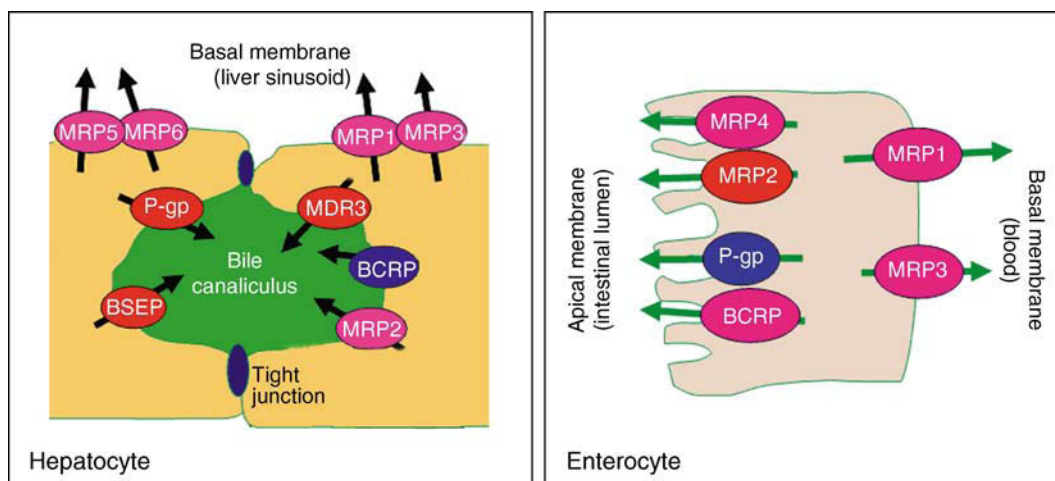
TMD and NBF (Fig. 1). Therefore, these transporters are termed *half transporter* (in contrast to *full transporter*); however, to achieve functional activity they have to form hetero- or homodimers.

Tissue Distribution and Expression

Although initially detected in cancer cell lines ABC-transporters show a wide tissue distribution. Several members of drug transporting ABC-proteins, for example, are highly expressed in physiological barriers such as the apical membrane of gut enterocytes, the endothelial cells of the blood–brain barrier or the maternal facing (apical) membrane of the placental syncytiotrophoblast. In all of these organs they protect sensitive tissues like brain or the growing fetus against potentially toxic compounds. In addition, ABC-transporter expression is highly abundant in hepatocytes (Fig. 2). Here, ABC-transporters are involved in detoxification of many endogenous and exogenous



ABC Transporters. Figure 1 Structure of ABCB1, ABCC1, and ABCG2 (NBF: *nucleotide binding fold*; TMD: *transmembrane domaine*). Modified according to ►www.iwaki-kk.co.jp/bio/specialedition/se02.htm).



ABC Transporters. Figure 2 ABC-transporter expression in hepatocytes and enterocytes (modified according to www.iwaki-kk.co.jp/bio/specialedition/se02.htm).

agents and are therefore expressed both in the canalicular and sinusoidal membrane. The canalicular expression is a prerequisite for biliary elimination. For example, the *bile salt export pump* (BSEP/ABCB11) is transporting bile salts, MRP2 (ABCC2) is involved in the elimination of organic anions like bilirubin-glucuronide or glutathione-conjugates and finally, P-gp (ABCB1) eliminates a wide variety of drugs into the bile. In contrast, other ABC-transporters like MRP1 (ABCC1) and 3 (ABCC3) are mainly located in the basal membrane of hepatocytes. They transport xenobiotics and several conjugates back to the blood and seem to be important under certain pathophysiological conditions, for example, hepatic expression of both transporters is enhanced during cholestasis, thereby protecting the hepatocytes against toxic bile acid concentrations by transport into the blood followed by increased renal elimination.

Various ABC-transporters are expressed in organs like heart, lung, pancreas, or cellular blood compounds. They may be important both for physiological processes and local drug concentrations. In this context, it is noteworthy that many of these transporters not only eliminate xenobiotic and toxic compounds from the cell, but also endogenous compounds. For example, MRP4, 5, and 8 (ABCC4, 5, and 11), which are expressed in many tissues and cancer cells, not only transport xenobiotics like nucleotide-based anticancer drugs but also the second messenger molecules cAMP and cGMP. Thereby, these transporters may play a role in regulating intra- and extracellular cyclic nucleotide concentrations.

ABC-Transporters and Disease

Based on their physiological function it is not surprising that genetic polymorphisms affecting expression and

function of ABC-proteins have been identified as the underlying mechanisms for some diseases. For example, mutations in the MRP2 (ABCC2) gene, which lead to the loss of this protein from the canalicular membrane of hepatocytes, are the mechanism of the **Dubin–Johnson Syndrome**. Here, the biliary elimination of MRP2 substrates like bilirubin and bilirubin-glucuronide is blocked; therefore the respective plasma levels are elevated leading to the disease. Another example is ABCC7, which is also called *cystic fibrosis transmembrane conductance regulator* (CFTR) and forms an anion channel in different tissues like the epithelial surfaces of the respiratory and intestinal tract. As its alias indicates, ABCC7 is involved in the pathogenesis of *cystic fibrosis*, because mutations in the ABCC7 gene associated with dysfunction or epithelial absence of the transporter are the underlying reason for the incorrect ion homeostasis, especially for chloride, which is the predominate anion transported by ABCC7 under physiological conditions.

Drugs

In this context, two aspects are important. First, many drugs are substrates of ABC-transporters and therefore these transporters might affect the bioavailability of these substances. Tissues like liver, intestine, and kidney exhibit high expression levels of different transport proteins. Therefore, substrates of these transporters may be intensively eliminated to the bile and urine or transported back to the intestine, thereby limiting oral bioavailability. Besides these pharmacokinetic important organs, ABC-transporters are expressed in target tissues of certain drugs. As already mentioned this point carries an unsolved problem in chemotherapy because many anticancer drugs are ABC-transporter substrates and tumor cells often show an enhanced

transporter expression and therefore MDR. However, this problem is not restricted to cancer therapy. For example, ABC-transporters are also expressed in the ►blood–brain barrier; thereby limiting the access of drugs to the brain. While this is useful for drugs like loperamide, a morphine-based drug against diarrhea, it might be a problem in the case of antipsychotic and antiepileptic drugs. A list of ABC-transporters and their substrates is given in Table 1.

Second, as already shown for P450 enzymes before, there is also a drug-interaction potential on the transporter level. The promoter regions of some ABC-transporter genes (e.g., P-gp) contain transcription factor binding sites like the *pregnane X receptor* (PXR), the *constitutive androstane receptor* (CAR), the *farnesoid X receptor* (FXR), the *steroid and xenobiotic receptor* (SXR) or the *peroxisome proliferator-activated receptor* (PPAR). Therefore, these proteins are not only regulated by endogenous compounds like bile acids or steroid hormones but also by therapeutic agents like phenobarbital, rifampicin, or dexamethasone. This regulation might be accompanied by an altered bioavailability of transporter substrates, when coadministered with these compounds. For example, the decreased bioavailability of digoxin, a P-gp substrate, after coadministration of rifampicin is due to an enhanced intestinal P-gp expression. On the other hand, many compounds are inhibitors of ABC-transporters (in the case of P-gp, for example, verapamil, ketoconazole, amiodarone, progesterone, indinavir, clarithromycin, cyclosporine, chlorpromazine, or methadone), which in turn leads to higher

plasma levels after coadministration of substrates for these transporters.

In addition, ABC-transporters demonstrate interindividual variability caused by genetic polymorphisms. Again, the *ABCB1* (P-gp) is the best characterized transporter in this field. Here, various synonymous and nonsynonymous polymorphisms as well as deletions and insertions have been described. Some of the nonsynonymous *single nucleotide polymorphisms* (SNPs) have already been shown to be associated with an altered transport activity of the protein. Interestingly, this observation has also been made for the C- to T-variant at position 3435, which represents the most frequent synonymous SNP of *ABCB1*. This C3435T polymorphism could be associated with an altered protein expression and function of P-gp, because individuals homozygous for this polymorphism show a significant lower intestinal P-gp expression. This finding was underlined by elevated digoxin plasma levels in patients homozygous for this SNP in comparison with the wild type. Recent data suggest that the altered protein expression and function of this variant may be due to the presence of a rare codon, which affects the timing of cotranslational folding and insertion of the protein into the membrane.

Taken together ABC-transporters represent a large family of proteins affecting the pharmacokinetic parameters of various drugs. Here, P-gp is currently the best characterized member and it may also be one of the most important ABC-transporters with regard to drug transport. However, it becomes more and more apparent that ABC-transporters act in a coordinated

ABC Transporters. Table 1 Substrates of ABC-transporters involved in multidrug resistance (MDR)

ABC-transporter	Transporter substrates
P-gp (ABCB1)	Verapamil, digoxin, mitoxantrone, vinblastine, doxorubicin, losartan, talinolol, cortisol, dexamethasone, colchicine, loperamide, domperidone, indinavir, erythromycin, tetracycline, itraconazole, cyclosporine, methotrexate, amitriptyline, phenobarbital, morphine, cimetidine, and others
MRP1 (ABCC1)	Glucuronides and sulfate conjugates of steroid hormones and bile salts, colchicine, doxorubicin, daunorubicin, epirubicin, folate, irinotecan, methotrexate, paclitaxel, vinblastine, vincristine, and others
MRP2 (ABCC2)	LTC ₄ , bilirubin-glucuronide, estradiol 17 β -glucuronide, dianionic bile salts, anionic conjugates, glutathione disulfide, and others
MRP3 (ABCC3)	Organic anions including bile salts
MRP4 (ABCC4)	PMEA, PMEG, ganciclovir, AZT, 6-mercaptopurine, thioguanine, methotrexate, cAMP, cGMP, estradiol 17 β -glucuronide, DHEAS, sulphated bile acids, glutathione, PGE ₁ , PGE ₂ , and others
MRP5 (ABCC5)	PMEA, PMEG, cladribine, gemcitabine, cytarabine, 5-FU, 6-mercaptopurine, thioguanine, cAMP, cGMP, glutathione, DNP-SG, CdCl ₂ , and others
BCRP (ABCG2)	Cisplatin, folate, methotrexate, mitoxantrone, topotecan, irinotecan, steroids (cholesterol, testosterone, progesterone), certain chlorophyll metabolites, and others

fashion with other detoxification systems like P450 enzymes and **▶uptake transporters**. In particular, P-glycoprotein and Cytochrome P450 3A4 are closely intertwined in terms of regulation and function. Thus, further reviews have to address the combined action of various systems.

- ▶MDR-ABC-Transporters
- ▶ATP-dependent K^+ Channel
- ▶Antracyclins

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ABPs

- ▶Actin Binding Proteins

Absence Epilepsy

Absence Epilepsies are a group of epileptic syndromes typically starting in childhood or adolescence and characterized by a sudden lack of attention and mild automatic movements for some seconds to minutes. Absence epilepsies are generalized, i.e. the whole neocortex shifts into a state of sleep-like oscillations.

- ▶Antiepileptic Drugs

Absorption

Absorption is defined as the disappearance of a drug from the site of administration and its appearance in the

blood (“central compartment”) or at its site of action. The main routes of administration are oral or parenteral (injection). After oral administration, a drug has to be taken up (is absorbed) from the gut. Here, the main site of absorption is the small intestine. In this case, only a portion of drug reaches the blood and arrives at its site of action.

- ▶Pharmacokinetics

Abstinence Syndrome

The abstinence syndrome (synonym, withdrawal symptom) is observed after withdrawal of a drug to which a person is addicted. For example, the abstinence syndrome after alcohol withdrawal is characterized by tremor, nausea, tachycardia, sweating and sometimes hallucinations.

- ▶Drug Addiction
- ▶Dependence

Abused Drugs

- ▶Drug Addiction/Dependence

Acadesine

5-Aminoimidazole-4-carboxamide ribonucleoside (also known as AICA riboside or AICAR). An adenosine analogue that is taken up into cells by adenosine transporters and converted by adenosine kinase to the monophosphorylated nucleotide form, ZMP. ZMP is an analogue of AMP that activates the AMP-activated protein kinase (AMPK), for which acadesine or AICAR can be used as a pharmacological activator.

- ▶AMP-activated Protein Kinase

ACE Inhibitors

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Synonyms

Angiotensin-converting enzyme inhibitors

Definition

Angiotensin converting enzyme (ACE) plays a central role in cardiovascular hemostasis. Its major function is the generation of angiotensin (ANG) II from ANG I and the degradation of bradykinin. Both peptides have profound impact on the cardiovascular system and beyond. ACE inhibitors are used to decrease blood pressure in hypertensive patients, to improve cardiac function, and to reduce work load of the heart in patients with cardiac failure.

Mechanism of Action

ACE inhibitors inhibit the enzymatic activity of ►angiotensin converting enzyme (ACE). This enzyme cleaves a variety of pairs of amino acids from the carboxy-terminal part of several peptide substrates. The conversion of ANG I to ANG II and the degradation of bradykinin to inactive fragments are considered the most important functions of ACE [1–3]. ACE inhibitors are nonpeptide analogues of ANG I. They bind tightly to the active sites of ACE, where they complex with a zinc ion and interact with a positively charged group as well as with a hydrophobic pocket. They competitively inhibit ACE with K_i values in the range between 10^{-10} and 10^{-11} [3].

Effects of ACE Inhibitors Mediated by the Inhibition of ANG II Generation

ANG II is the effector peptide of the renin–angiotensin system [1, 2]. ANG II is one of the most potent vasoconstrictors, facilitates norepinephrine release, stimulates aldosterone production, and increases renal sodium retention. In addition, ANG II is considered to be a growth factor, stimulating proliferation of various cell types. The actions of ANG II are mediated through two ►angiotensin receptors, termed AT_1 and AT_2 . Most of the cardiovascular functions of ANG II are mediated through the AT_1 receptor.

In some patients with hypertension and in all patients with cardiac failure, the renin–angiotensin system is activated to an undesired degree, burdening the heart. The consequences of diminished ANG II generation by ACE inhibitors are multiple. In patients with hypertension, blood pressure is reduced as a result

of (i) decreased peripheral vascular resistance, (ii) decreased sympathetic activity, and (iii) reduced sodium and water retention. In patients with cardiac failure, cardiac functions are improved as a result of (i) reduced sodium and water retention (preload and afterload reduction), (ii) diminished total peripheral resistance (afterload reduction), and (iii) reduced stimulation of the heart by the sympathetic nervous system. A reduction of cardiac hypertrophy appears to be another desired effect of ACE inhibitors. It is mediated at least partially by the reduction of intracardiac ANG II levels. ACE inhibitors furthermore protect the heart from arrhythmia during reperfusion after ischemia, and improve local blood flow and the metabolic state of the heart. These effects are largely mediated by Bradykinin (see below).

In the vasculature, ANG II not only increases contraction of smooth muscle cells, but is also able to induce vascular injury. This can be prevented by blocking ►NF κ B activation [3] suggesting a link between ANG II and inflammation processes involved in the pathogenesis of arteriosclerosis (see below). Thus, ACE inhibitors not only decrease vascular tone but probably also exert vasoprotective effects.

In the kidney, ANG II reduces renal blood flow and constricts preferentially the efferent arteriole of the glomerulus with the result of increased glomerular filtration pressure. ANG II further enhances renal sodium and water reabsorption at the proximal tubulus. ACE inhibitors thus increase renal blood flow and decrease sodium and water retention. Furthermore, ACE inhibitors are nephroprotective, delaying the progression of glomerulosclerosis. This also appears to be a result of reduced ANG II levels and is at least partially independent from pressure reduction. On the other hand, ACE inhibitors decrease glomerular filtration pressure due to the lack of ANG II-mediated constriction of the efferent arterioles. Thus, one important undesired effect of ACE inhibitors is impaired glomerular filtration rate and impaired kidney function.

Another effect of ANG II is the stimulation of ►aldosterone production in the adrenal cortex. ANG II increases the expression of steroidogenic enzymes, such as aldosterone synthase and stimulates the proliferation of the aldosterone-producing zona glomerulosa cells. Aldosterone increases sodium and water reabsorption at the distal tubuli. More recently it has been recognized that aldosterone is a fibrotic factor in the heart. ACE inhibitors decrease plasma aldosterone levels on a short-term scale, thereby not only reducing sodium retention but also preventing aldosterone-induced cardiac fibrotic processes. On a long-term scale, however, patients with cardiac failure exhibit high aldosterone levels even when taking ACE inhibitors.

In this context, it is important to note that circulating ANG II levels do not remain reduced during long-term treatment with ACE inhibitors. This is likely the result of activation of alternative, ACE-independent pathways of ANG II generation. The protective effects of ACE inhibitors on a long-term scale, therefore, are not explained by a reduction of circulating ANG II levels. They are either unrelated to inhibition of ANG II generation, or a result of the inhibition of local generation of ANG II. Indeed, due to the ubiquitous presence of ACE in endothelial cells, large amounts of ANG II are generated locally within tissues such as kidney, blood vessels, adrenal gland, heart, and brain, and exert local functions without appearing in the circulation [2]. Membrane-bound endothelial ACE, and consequently local ANG II generation, has been proved to be of greater significance than ANG II generated in plasma by the circulating enzyme. Experimental evidence also indicates that plasma ACE may in fact not be relevant to blood pressure control at all.

Effects of ACE Inhibitors Mediated by the Inhibition of Bradykinin Degradation

Kinins are involved in blood pressure control, regulation of local blood flow, vascular permeability, sodium balance, pain, inflammation, platelet aggregation and coagulation. Bradykinin also exerts antiproliferative effects [4]. In plasma, bradykinin is generated from high molecular weight (HMW) kininogen, while in tissues lys-bradykinin is generated from HMW and low molecular weight (LMW) kininogen. Several effects of bradykinin are explained by the fact that the peptide potentially stimulates the NO-pathway and increases prostaglandin synthesis in endothelial cells. In smooth muscle cells and platelets, NO stimulates the soluble guanylate cyclase, which increases cyclic GMP that in turn activates protein kinase G. As a consequence, vascular tone and subsequently systemic blood pressure is decreased, local blood flow is improved, and platelet aggregation is prevented.

ACE inhibitors inhibit the degradation of bradykinin and potentiate the effects of bradykinin by about 50–100-fold. The prevention of bradykinin degradation by ACE inhibitors is particularly protective for the heart. Increased bradykinin levels prevent postischemic reperfusion arrhythmia, delays manifestations of cardiac ischemia, prevents platelet aggregation, and probably also reduces the degree of arteriosclerosis and the development of cardiac hypertrophy. The role of bradykinin and bradykinin-induced NO release for the improvement of cardiac functions by converting enzyme inhibitors has been demonstrated convincingly with use of a specific bradykinin receptor antagonist and inhibitors of NO-synthase.

In the kidney, bradykinin increases renal blood flow, whereas glomerular filtration rate remains unaffected.

Bradykinin stimulates natriuresis and, through stimulation of prostaglandin synthesis, inhibits the actions of antidiuretic hormone (ADH), thereby inhibiting water retention. Bradykinin further improves insulin sensitivity and cellular glucose utilization of skeletal muscle cells in experimental models. This, however, appears not to be relevant in the clinical context.

Bradykinin exerts its effects via B₁ and B₂ receptors. The inhibition of bradykinin degradation by ACE inhibitors compensatory leads to increased conversion of bradykinin to des Arg-9-bradykinin by kininase I. This peptide still has strong vasodilatory properties and a high affinity to the B₁ receptor. The clinical relevance of this aspect is not clear. The cardioprotective effects of bradykinin are mediated via B₂ receptors, since they can be blocked by a specific B₂ receptor antagonist [4]. On the other hand, kinins increase vascular permeability with the consequence of edema, exhibit chemotactic properties with the risk of local inflammation and they are involved in the manifestation of endotoxic shock. Increased bradykinin levels are thus thought to cause some of the undesired effects observed with ACE inhibitors, such as cough, allergic reactions, and anaphylactic responses, for instance angioneurotic edema [5].

Clinical Use (Including Side Effects)

ACE inhibitors are approved for the treatment of hypertension and cardiac failure [5]. For cardiac failure, many studies have demonstrated increased survival rates independently of the initial degree of failure. They effectively decrease work load of the heart as well as cardiac hypertrophy and relieve the patients symptoms. In contrast to previous assumptions, ACE inhibitors do not inhibit aldosterone production on a long-term scale sufficiently. Correspondingly, additional inhibition of aldosterone effects significantly reduces cardiac failure and increases survival even further in patients already receiving diuretics and ACE inhibitors. This can be achieved by coadministration of spironolactone, which inhibits binding of aldosterone to its receptor.

In the treatment of hypertension, ACE inhibitors are as effective as diuretics, β -adrenoceptor antagonists, or calcium channel blockers in lowering blood pressure. However, increased survival rates have only been demonstrated for diuretics and β -adrenoceptor antagonists. ACE inhibitors are approved for monotherapy as well as for combinational regimes. ACE inhibitors are the drugs of choice for the treatment of hypertension with renal diseases, particularly diabetic nephropathy, because they prevent the progression of renal failure and improve proteinuria more efficiently than the other drugs.

More than 15 ACE inhibitors are presently available. They belong to three different chemical classes: sulfhydryl compounds such as captopril, carboxyl compounds such as enalapril, and phosphorus compounds

such as fosinopril. Sulfhydryl compounds exert more undesired, but also desired effects, since they additionally interact with endogenous SH groups. For instance, these compounds may potentiate NO-actions or act as scavengers for oxygen-derived free radicals. Carboxyl compounds are in general more potent than captopril. Phosphorous compounds are usually characterized by the longest duration of action.

Most ACE inhibitors are prodrugs, with the exceptions of captopril, lisinopril, and ceranapril. Prodrugs exert improved oral bioavailability, but need to be converted to active compounds in the liver, kidney, and/or intestinal tract. In effect, converting enzyme inhibitors have quite different kinetic profiles with regard to half time, onset and duration of action, or tissue penetration.

In general, ACE inhibitors at the doses used to date are safe drugs. In contrast to many antihypertensive drugs, ACE inhibitors do not elicit a reflex tachycardia and do not influence lipid or glucose metabolism in an undesired manner. Glucose tolerance is even increased. Most undesired effects are class-specific and related to the inhibition of ACE. Less dangerous, but often bothersome, are dry cough, related to increased bradykinin levels and loss of taste or impaired taste. The more severe undesired effects are hypotension, hyperkalemia, and renal failure, but those can be easily monitored and appropriately considered. The risk for hypotension increases in combination with diuretics, particularly when ACE inhibitors are initiated in patients who already receive diuretics. The risk of hyperkalemia increases with coadministration of spironolactone and the risk of renal failure is higher in volume-depleted patients or those already exhibiting impaired renal function. Seldom (0.05%) the development of angioneurotic edema occurs (usually) during the first days of treatment and is life threatening. Allergic responses and angioneurotic edema are related to bradykinin. Recently, specific AT₁ receptor antagonists have become available and are used in the management of hypertension and are presently tested for use in cardiac failure. They are believed not to exhibit the bradykinin-related undesired effects. Indeed, undesired effects of AT₁ receptor antagonists are lower than seen with ACE inhibitors. On the other hand, AT₁ receptor antagonists are probably less effective since the patients do not profit from the cardioprotective effects of bradykinin. Studies comparing the effects of ACE inhibitors with AT₁ receptor antagonists are presently underway. ACE inhibitors are contraindicated in pregnancy (risk of abortion, acute renal failure of the newborn) and patients with bilateral stenosis of the renal artery. Special caution should be taken if patients have autoimmune diseases.

- ▶ Blood Pressure Control
- ▶ Renin–Angiotensin–Aldosterone System

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ACE2

- ▶ Angiotensin-converting Enzyme-2

Acetyl β-methylcholinesterase

- ▶ Cholinesterase

Acetylcholine

Acetylcholine (ACh) is an ester of acetic acid and choline with the chemical formula $\text{CH}_3\text{COOCH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3$. ACh functions as a chemical transmitter in both the peripheral nervous system (PNS) and central nervous system (CNS) in a wide range of organisms, humans included. Neurotransmitter involved in behavioral state control, postural tone, cognition and memory, and autonomous parasympathetic (and preganglionic sympathetic) nervous system.

- ▶ Cholinesterases
- ▶ Emesis

- Orexins
- Muscarinic Receptors
- Nicotinic Receptors

Acetylcholine Hydrolase

- Cholinesterase

Acetylcholinesterase

Acetylcholine serves as a neurotransmitter. Removal of acetylcholine within the time limits of the synaptic transmission is accomplished by acetylcholinesterase (AChE). The time required for hydrolysis of acetylcholine at the neuromuscular junction is less than a millisecond (turnover time is 150 μ s) such that one molecule of AChE can hydrolyze 6×10^5 acetylcholine molecules per minute. The K_m of AChE for acetylcholine is approximately 50–100 μ M. AChE is one of the most efficient enzymes known. It works at a rate close to catalytic perfection where substrate diffusion becomes rate limiting. AChE is expressed in cholinergic neurons and muscle cells where it is found attached to the outer surface of the cell membrane.

- Neurotransmitter Transporters
- Cholinesterase

Acetylthiocholinesterase

- Cholinesterase

Acetyltransferase

Acetyltransferase is an enzyme that catalyses the transfer of an acetyl group from one substance to another.

- Histon Acetylation

N-Acetyltransferases

N-Acetyltransferases (NATs) catalyze the conjugation of an acetyl group from acetyl-CoA on to an amine, hydrazine or hydroxylamine moiety of an aromatic compound. NATs are involved in a variety of phase II-drug metabolizing processes. There are two isozymes NAT I and NAT II, which possess different substrate specificity profiles. The genes encoding NAT I and NAT II are both multi-allelic. Especially for NAT II, genetic polymorphisms have been shown to result in different phenotypes (e.g., fast and slow acetylators).

- Pharmacokinetics
- Pharmacogenetics

α 1-Acid Glycoprotein

One of the plasma proteins which is mainly responsible for the plasma protein binding of drugs. Its level is known to be elevated in some pathological states, such as inflammation.

- Drug Interaction

Acidosis

State of deviation of plasma pH (systemic acidosis) or tissue extracellular pH (tissue or local acidosis) from normal (ca. pH 7.4) towards lower values. Deviation of 0.1 pH units is significant. Systemic acidosis can be caused by lung or kidney failure. Local acidosis can be the consequence of injury, inflammation, or tumor growth, due to disruption of blood supply. Local acidosis is normally associated with hypoxia.

- Proton-Sensing GPCRs

ACPD

ACPD (1-aminocyclopentane-1,3-dicarboxylic acid) is a selective agonist for metabotropic glutamate (mGlu)

receptors. Within the 4 stereoisomers, 1S,3R-ACPD activates group-I and group-II mGlu receptors as well as some group-III receptors (mGlu8) at higher concentrations. The 1S,3S-ACPD isomer is one of the first selective group-II mGlu receptor agonists described. These molecules have been widely used to identify the possible physiological functions of mGlu receptors.

► **Metabotropic Glutamate Receptors**

ACTH

Adrenocorticotrope Hormone.

► **Glucocorticoid Receptors**

Actin

► **Cytoskeleton**

Actin Binding Proteins

By binding to F-actin, actin binding proteins (ABPs) stabilize F-actin or regulate its turnover. Known ABPs are proteins such as α -actinin, talin, tensin, filamin, nexilin, fimbrin, and vinculin.

► **Cytoskeleton**

Actin Filaments

► **Cytoskeleton**

Action Potential

An Action Potential is a stereotyped (within a given cell) change of the membrane potential from a resting

(intracellular negative) value to a depolarized (intracellular positive) value and then back to the resting value. The durations of Action Potentials range from a couple of milliseconds in nerve cells to hundreds of milliseconds in cardiac cells. Action Potentials may be propagated along very elongated cells (skeletal muscles, axons of neurons, etc) or from one cell to another via electrical gap junctions (e.g. in cardiac tissue).

- **Inwardly Rectifying K^+ Channels**
- **Voltage-dependent Na^+ Channels**
- **Antiarrhythmic Drugs**

Activated Partial Thromboplastin Time

Activated partial thromboplastin time (aPTT) is a coagulation assay, which measures the time for plasma to clot upon activation by a particulate substance (e.g., kaolin) in the presence of negatively charged phospholipids.

► **Anticoagulants**

Activator Protein-1

Activator Protein-1 (AP1) comprises transcriptional complexes formed by dimers of members of the Fos, Jun, and ATF family of transcription factors. These proteins contain basic leucine zipper domains that mediate DNA binding and dimerization. They regulate many aspects of cell physiology in response to environmental changes.

► **NFAT Family of Transcription Factors**

Active Site

Active site of an enzyme is the binding site where catalysis occurs. The structure and chemical properties of the active site allow the recognition and binding of the substrate. The active site is usually a small pocket at the surface of the enzyme that contains residues responsible

for the substrate specificity (charge, hydrophobicity, and steric hindrance) and catalytic residues which often act as proton donors or acceptors or are responsible for binding a cofactor such as pyridoxal, thiamine, or NAD. The active site is also the site of inhibition of enzymes.

Active Transport

Permeation of a drug through biological membranes against the electrochemical gradient. This type of drug transport requires energy produced by intracellular metabolic processes.

- ▶ Drug Interaction
- ▶ ABC Transporters
- ▶ MDR-ABC Transporters

Active Transporters

Active Transporters use the energy of ATP for vectorial transport through a biological membrane against concentration gradient of the transported substrate.

- ▶ ABC Transporters
- ▶ MDR-ABC Transporters

Activins

Activins are growth and differentiation factors belonging to the transforming growth factor- β superfamily. They are dimeric proteins, consisting of two inhibin- β subunits. The structure of activins is highly conserved during vertebrate evolution. Activins signal through type I and type II receptor serine/threonine receptor kinases. Subsequently downstream signals such as Smad proteins are phosphorylated. Activins are present in many tissues of the mammalian organism, where they function as autocrine and/or paracrine regulators of various physiological processes, including reproduction. In the hypothalamus, activins are thought to stimulate the release of gonadotropin-releasing hormone. In the pituitary, activins increase follicle-stimulating hormone secretion and up-regulate gonadotropin-releasing hormone receptor expression. In

the ovaries, activins regulate processes such as folliculogenesis, steroid hormone production and oocyte maturation. During pregnancy, activin-A is also involved in the regulation of placental functions.

- ▶ Receptor Serine/Threonine Receptor Kinase
- ▶ Transforming Growth Factor- β Superfamily

Acute Phase Reactants

Acute phase reactants (e.g., C-reactive protein) are proteins that increase during inflammation and are deposited in damaged tissues. They were first discovered in the serum, but are now known to be involved in inflammatory processes in the brain (e.g., found in the brain of Alzheimer patients and associated with amyloid plaques).

- ▶ Inflammation
- ▶ Neurodegeneration

Acyl-CoA

Acyl-CoAs are the activated intermediates of fatty acid metabolism formed by the condensation of fatty acids with Coenzyme A.

- ▶ Lipid Modifications
- ▶ Fatty Acid Transporters

Acyl-CoA Synthetase

Acyl-CoA synthetases are enzymes (i.e., ligases) that convert fatty acid molecules into acyl-Coenzyme A molecules for their subsequent oxidation.

- ▶ Fatty Acid Transporters
- ▶ Lipid Modification

Adaptive Immunity

The adaptive or specific arm of the immune system consists of T- and B-lymphocytes and antibodies. T- and

B-cells carry antigen receptors that are generated by random genetic rearrangement during the ontogeny of lymphocytes in the bone marrow (B cells) or the thymus (T-cells). The hallmarks of adaptive immunity are the improved and specific defenses by T and B memory cells and antibodies after repeated exposure (immunological memory) to the eliciting antigen.

► Immune Defense

Adaptor Proteins

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Synonyms

Scaffold; Docking protein; Anchoring protein

Definition

Adaptor proteins are multi-domain proteins (Fig. 1) that interact with components of signaling pathways [1]. As a consequence of these interactions, adaptor proteins are able to regulate signaling events within the cell, providing spatiotemporal control and specificity, and influencing how a cell responds to a particular stimulus.

Basic Mechanisms

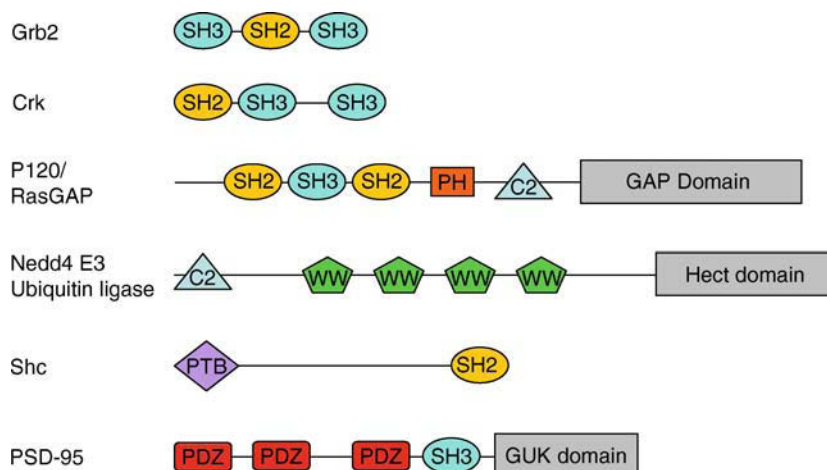
Adaptor proteins function by simultaneously interacting with multiple components of a signaling pathway (Fig. 2). In order to be able to bind to more than one target protein at the same time, adaptor proteins contain at least two specific protein-protein interaction domains. These domains recognize specific motifs in the target proteins and can act completely independently, like beads on a string, or interact with another domain within the same molecule. Such intramolecular interactions can regulate the ability of each domain to bind to its target.

Adaptor Protein Function

In their simplest form, adaptor proteins perform a straightforward function: the formation of multi-protein complexes. However, they often provide more than a static scaffold support for signaling components, instead enabling dynamic regulation to control propagation of pathways and networks. Consequently, adaptor proteins can act as signaling modules, directing propagation of the pathway, influencing downstream events and even modifying the cellular response to a specific stimulus. Some of the different roles played by adaptor proteins are described below. These functions are not mutually exclusive and more than one of these roles can be performed by a particular adaptor protein at one time.

Assembly of Signaling Complexes

This is perhaps the simplest function provided by adaptor proteins and involves bringing together



Adaptor Proteins. Figure 1 Adaptor protein domains. A scheme of the domain structures of some well-characterized adaptor proteins is shown. Descriptions of domain characteristics are in main text except: C2, binds to phospholipids; GTPase activating protein (GAP) domain, inactivates small GTPases such as Ras; Hect domain, enzymatic domain of ubiquitin ligases and GUK domain, guanylate kinase domain. For clarity, not all domains contained within these proteins are shown.

individual components of a pathway. These complexes promote propagation, and often amplification, of the signal. Examples of this are found in the ►MAP kinase cascade pathway, where the adaptor proteins IQGAP1 and kinase suppressor of Ras-1 bind to multiple ►kinases. Consequently, they enable efficient signaling from one kinase to the next.

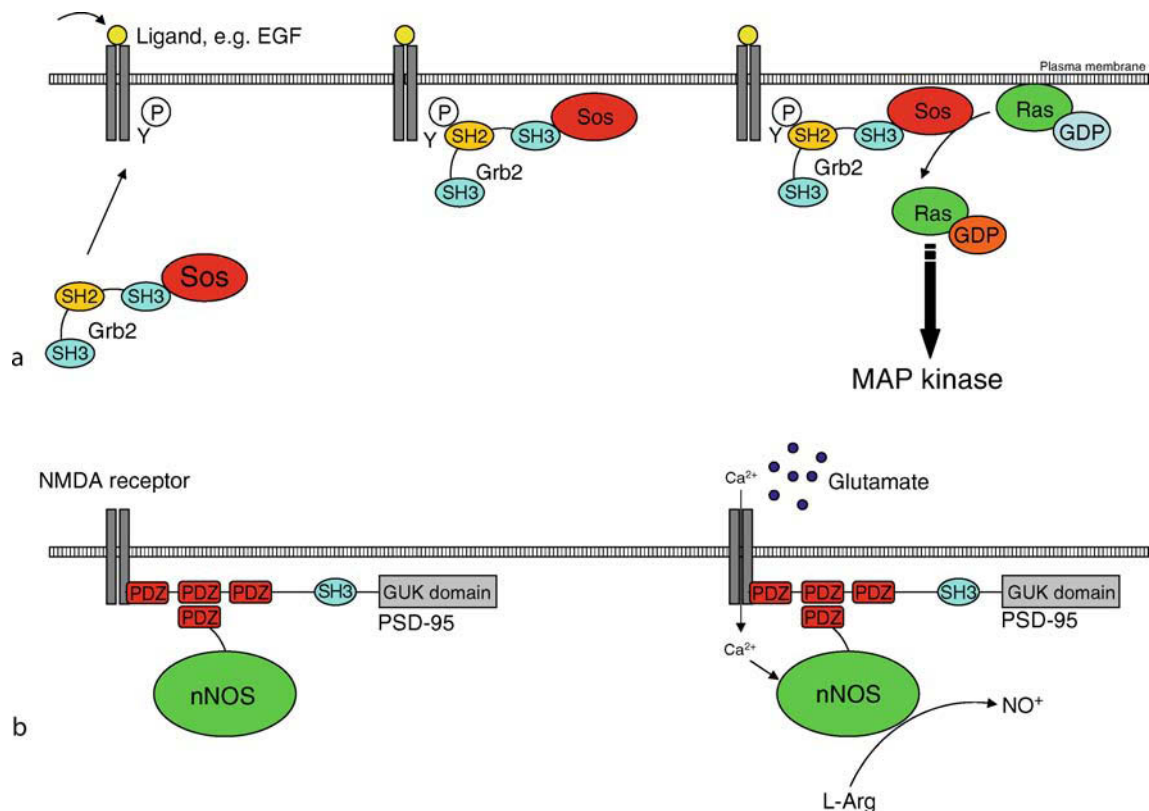
Spatial Regulation

Adaptor proteins can assemble the complexes in particular subcellular compartments. For example, following activation of a ligand-bound receptor, adaptor proteins can localize downstream signaling targets to the intracellular domains of the receptor (Fig. 2a), thereby facilitating propagation of the signal through the cell. Note that signaling events occur in all cell

organelles and subcellular compartments and adaptor proteins appear to function throughout the cell. Through their ability to localize signaling targets to specific subcellular compartments, adaptor proteins not only facilitate signaling events, but also influence how these signals are interpreted by the cell, and consequently the cellular response. For example, in the MAP kinase pathway, signaling from the plasma membrane activates different signaling components to those activated when the signal originates from the Golgi.

Temporal Regulation

The duration of signaling influences how a cell responds to a particular stimulus. For example, brief activation of the MAP kinase cascade in the neuronal cell line, PC12, results in proliferation, while sustained



Adaptor Proteins. Figure 2 Examples of adaptor protein function. Selected examples of how adaptor proteins facilitate signaling are shown. (a) Growth factor signaling. Many growth factor receptors contain an intrinsic tyrosine kinase. Following stimulation by cognate ligand binding, the intracellular domains of the transmembrane receptor autophosphorylate on key tyrosine (Y) residues. The SH2 domain of the adaptor protein Grb-2 associates with the phosphorylated tyrosine residues on the receptor, while the SH3 domain binds to the Ras activator Sos. Consequently, Sos is localized to the receptor and the plasma membrane where it activates the small GTPase Ras, which results in activation of the mitogen-activated protein (MAP) kinase cascade. By this mechanism, Grb-2 facilitates the transduction of an extracellular stimulus to an intracellular signaling pathway. (b) The adaptor protein PSD-95 associates through one of its three PDZ domains with the N-methyl-D-aspartic acid (NMDA) receptor. Another PDZ domain associates with a PDZ domain from neuronal nitric oxide synthase (nNOS). Through its interaction with PSD-95, nNOS is localized to the NMDA receptor. Stimulation by glutamate induces an influx of calcium, which activates nNOS, resulting in the production of nitric oxide.

MAP kinase signaling promotes differentiation of PC12 cells. Adaptor proteins are able to regulate the time course of signaling events, and therefore the cellular outcome.

Activation of Signaling Components

The binding of an adaptor protein may activate the target molecule. An example of this is the adaptor protein insulin receptor substrate-1 (IRS-1), which is activated during insulin signaling. Following binding by insulin, the insulin receptor (which contains an intrinsic tyrosine kinase) catalyzes phosphorylation of selected tyrosine residues on IRS-1. A subset of these phosphorylated tyrosines act as docking sites for SH2 (see below) domains on p85, the regulatory subunit of phosphoinositide 3-kinase. As a consequence of the interaction between IRS-1 and p85, phosphoinositide 3-kinase is activated.

Inactivation of Kinases

►A-kinase anchoring proteins (AKAPs) are a well-studied class of adaptor proteins that regulate protein kinase A (PKA) signaling [2]. PKA is activated in response to 3′/5′-cyclic-adenosine monophosphate (cAMP). ►AKAPs provide spatiotemporal specificity for PKA activity by forming multi-protein complexes and localizing them to the appropriate subcellular compartment. These complexes contain kinases and phosphatases, as well as phosphodiesterases, which catalyze the hydrolysis of cAMP to AMP. Therefore AKAPs anchor both positive and negative regulators of PKA signaling.

Sequestering Signaling Components

Specificity in signal transduction is also achieved by selective separation of signaling components. By associating with specific proteins and bringing them together, adaptor proteins can determine how the pathway propagates through the cell. This is important for ►Ras signaling, for example. Ras, which can be activated in response to large number of growth factors and signaling cues, regulates multiple pathways. Therefore, by sequestering specific proteins, adaptor proteins ensure that a particular stimulus activates the appropriate pathway(s). Sequestration of proteins also enables adaptor proteins to negatively regulate signaling. An example of this can be found in T cell activation. The adaptor protein, c-Cbl, sequesters the tyrosine kinase, Syk, preventing recruitment to IgE receptors. Consequently, T cell activation is attenuated.

Domains

In order to bind to target proteins, adaptor proteins contain protein-protein interaction domains which recognize specific target motifs (Fig. 1). Through combinations of these domains, adaptor protein can interact with multiple

target proteins, potentially forming large signaling complexes. Whilst many different protein binding domains have been identified, adaptor proteins often utilize the well characterized domains described below.

Src Homology 2 (SH2) and Src Homology 3 (SH3)

►SH2 domains are common protein modules that recognize short motifs containing a tyrosine residue that has been phosphorylated by a tyrosine kinase. Other residues outside of this motif provide specificity to determine which SH2 domain-containing protein associates with that particular site. ►SH3 domains bind to polyproline motifs with the sequence PXXP. These two domains are often found independently in adaptor proteins such as ►Src-homologous and collagen (Shc) and ►PSD-95 (Fig. 1). However, the SH2/SH3 adaptors ►Crk and ►Grb-2 contain both SH2 and SH3 domains (Fig. 1). In these proteins, the SH2 domain recognizes a binding motif in activated transmembrane receptors, while the SH3 recognizes other signaling proteins, such as the Ras activator ►Sos (Fig. 2). Consequently, these proteins couple an activated receptor to signaling components downstream, thereby facilitating signal propagation.

Post Synaptic Density Protein/Drosophila Disc Large Tumor Suppressor/Zonula Occludens-1 Protein (PDZ)

►PDZ domains bind to short peptide motifs at the C-terminal end of target proteins, and are particularly important in spatial organization of receptors and ion channels [3]. Many adaptor proteins contain multiple PDZ domains, which have important implications for their functions. By interacting with subunits from different receptors, adaptor proteins containing multiple PDZ domains can promote formation of homogenous receptor complexes. This clustering can be further enhanced through the ability of PDZ domains to self-associate, enabling oligomerization of the adaptor proteins. Conversely, individual PDZ domains can associate with different target proteins, enabling the formation of large heterogeneous complexes of proteins. Examples of PDZ containing adaptor proteins include ►PSD-95 (Fig. 1), Lim kinase and membrane-associated guanylate kinases (MAGUKs).

Phosphotyrosine-binding (PTB)

►PTB domains recognize small peptides containing a phosphotyrosine, usually with the consensus sequence, NPXpY. Some PTB-containing proteins, such as Numb, are able to bind to the consensus peptide in the absence of phosphorylated tyrosine, suggesting phosphotyrosine is dispensable for the function of certain PTB domains. Hydrophobic residues N-terminal to the phosphotyrosine provide some specificity of target and distinction from SH2 domains. PTB domains appear to be particularly important in docking

proteins to activated receptors. Examples of PTB containing proteins include Numb, IRS-1 and Shc [4] (Fig. 1).

WW

► **WW** domains (named after the one letter abbreviation for the amino acid tryptophan) are small regions of around 30 residues, which, like SH3 domains, bind to polyproline sequences. These sequences often contain the consensus sequence PPXY or PPLP. Examples of proteins that contain WW domains include Nedd4 E3 ubiquitin ligase (Fig. 1) and IQGAP1.

Pleckstrin Homology (PH)

► **PH** domains consist of about 120 amino acid residues. They do not interact with other proteins, but associate with specific polyphosphoinositides. Consequently, PH domains appear to be important for localizing target proteins to the plasma membrane. Examples of PH domain-containing proteins include phospholipase C and p120/RasGAP (Fig. 1).

Regulation of Adaptor Proteins

The interactions between adaptor proteins and their targets are often regulated (see below). By these mechanisms, specific signals are able to control which adaptor proteins, and consequently which target proteins, are recruited to a particular signaling complex. Common methods of adaptor protein regulation are described here.

Phosphorylation

Phosphorylation is a common method of regulation. As described above, SH2 domains bind to phosphorylated tyrosine residues. Conversely, phosphorylation of serines and threonines proximal to SH3 and PDZ domains uncouples them from their target motifs. Therefore modulation of protein kinase activity in cells regulates interactions between adaptor proteins and their target proteins.

Chemical Regulation

Cellular messengers, such as calcium, also regulate adaptor protein function. The adaptor protein IQGAP1 binds multiple members of the MAP kinase cascade, including B-Raf, MAPK/extracellular-regulated kinase (MEK) and extracellular-regulated kinase (ERK). Binding of calcium to its effector, calmodulin, increases the association of calmodulin with IQGAP1 and inhibits the interaction with B-Raf.

Intramolecular Interactions

Another way in which the function of adaptor proteins is regulated is through modulation of intramolecular interactions. Often one domain will bind to another domain in the same adaptor molecular, preventing

further interactions with other proteins. An example of this is found in the adaptor protein, Crk. Crk contains an N-terminal SH2 domain, followed by two SH3 domains. The SH3 domains are separated by a linker region containing a tyrosine residue. When Crk is phosphorylated by a tyrosine kinase, such as Abl, the intrinsic SH2 domain binds to the phosphotyrosine, attenuating Crk signaling activity.

Conformational Changes

Changing the conformation of adaptor proteins can also alter their function. p130Cas, which is a target for ► **Src kinase**, serves as an example. The central region of p130Cas contains multiple ► **Src** phosphorylation sites that, when phosphorylated, promote the binding and recruitment of other target proteins. Under resting conditions, these phosphorylation sites are hidden due to the folded conformation of p130Cas. When mechanical stress is applied to the cell, this central region is stretched exposing the Src phosphorylation sites. This alteration in conformation results in increased phosphorylation of p130Cas and recruitment of the adaptor protein ► **CrkII**, leading to activation of the Ras family GTPase, Rap1.

Pharmacological Intervention

Adaptor proteins are attractive targets for the design of new therapies against diseases in which signaling pathways are deregulated. For example, many cancers and inflammatory disorders display hyperactive MAP kinase signaling. This may be due to increased growth factor/cytokine stimulation or increased intracellular kinase activity. Adaptor proteins play well-established roles in controlling MAP kinase activation, and so provide a potential target for novel therapies [5]. For example, Grb-2 has been the focus of research to identify compounds which target either its SH2 or SH3 domains. The importance of Grb-2 in the activation of Ras, and therefore stimulation of the MAP kinase pathway, suggests compounds that inhibit Grb-2 function could potentially be useful in the treatment of many cancers.

At present no compounds targeting adaptor proteins have been approved for clinical use.

Therapies for treatment of diseases caused by hyperactive intracellular signaling may utilize inhibitors targeted against a specific kinase. However, this approach has several problems, such as inhibition of other non-target kinases and inhibition of signaling events not related to the disease. Consequently, the use of kinase inhibitors is not always effective and often produces side effects. However, as described above, adaptor proteins provide specificity to signaling pathways. Therefore, disrupting adaptor protein function may allow more specific targeting of the aberrant cellular response which contributes to the disease, such

as cell proliferation or cytokine production. By selectively interfering with adaptor interactions, these agents are likely to have fewer side effects and increased efficacy.

► A Kinase Anchoring Proteins (AKAPs)

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Addiction

- Dependence
- Drug Addiction

Addison's Disease

An endocrine disorder first described by the British Physician Thomas Addison in the mid 1800's. The adrenal glands fail to produce sufficient amounts of glucocorticoid hormones (cortisol) and sometime mineralocorticoid (aldosterone). If left untreated it is life-threatening, the patient will show muscle weakness, hyperpigmentation and even depression. Typical treatment is hydrocortisone replacement therapy.

- Melanocortin

Additive Interaction

Interaction in which the combined effect is the sum of the effects of each drug administered separately.

- Drug Interaction

Adenosine

Adenosine is produced by many tissues, mainly as a by-product of ATP breakdown. It is released from neurons, glia and other cells, possibly through the operation of the membrane transport system. Its rate of production varies with the functional state of the tissue and it may play a role as an autocrine or paracrine mediator (e.g. controlling blood flow). The uptake of adenosine is blocked by dipyridamole, which has vasodilatory effects. The effects of adenosine are mediated by a group of G protein-coupled receptors (the $G_{i/o}$ -coupled A_1 - and A_3 receptors, and the G_s -coupled A_{2A} -/ A_{2B} receptors). A_1 receptors can mediate vasoconstriction, block of cardiac atrioventricular conduction and reduction of force of contraction, bronchoconstriction, and inhibition of neurotransmitter release. A_2 receptors mediate vasodilatation and are involved in the stimulation of nociceptive afferent neurons. A_3 receptors mediate the release of mediators from mast cells. Methylxanthines (e.g. caffeine) function as antagonists of A_1 and A_2 receptors. Adenosine itself is used to terminate supraventricular tachycardia by intravenous bolus injection.

- Purinergic System
- Adenosine Receptors
- Sterol Transporters

Adenosine Receptors

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Synonyms

Ado receptors; A receptors; P1 receptors

Definition

Extracellular adenosine acts through a class of ► **G protein-coupled receptors** (GPCRs), defined across mammalian species as A_1 , A_{2A} , A_{2B} , and A_3 ARs (adenosine receptors). Adenosine has a cytoprotective role in the body, both in the periphery and in the central nervous system. Following binding of adenosine, or another naturally occurring ► **agonist**, the receptor

interacts with heterotrimeric G proteins to stimulate or inhibit downstream signaling cascades.

Basic Characteristics

The purine nucleoside adenosine, as a natural local modulator of cell action, increases the ratio of oxygen supply to demand, suppresses excessive inflammation, and promotes tissue protection against apoptosis or ischemic damage. ARs also have effects on proliferation and differentiation. Nearly every cell type in the body expresses one or more of these receptors, indicating the fundamental nature of adenosine as a cytoprotective mediator. In the cardiovascular system, ARs present on myocardial, vascular, and inflammatory cells respond to the stress of ischemia and other damaging conditions. Adenosine has been termed a “retaliatory” metabolite of ATP, in that its extracellular level rises in response to excessive energy demand in relation to the available energy supply, i.e., utilization of intracellular ATP. Adenosine aids in correcting this imbalance between energy supply and demand by causing vasodilation, increasing vascular integrity and angiogenesis, and counteracting the lethal effects of prolonged ischemia on cardiac myocytes. In the brain, activation of presynaptic A₁ARs suppresses the release of excitatory neurotransmitters and counteracts excitotoxicity by both presynaptic and postsynaptic mechanisms.

The affinity and selectivity of nucleosides as adenosine ►agonists has been extensively explored, resulting in thousands of selective agonists, of which many are useful in pharmacological studies [1]. Selected agonists of A₁, A_{2A}, and A₃ receptors are shown in Fig. 1. Highly selective ►antagonists of A₁, A_{2A}, A_{2B}, and A₃ receptors have been reported both as research tools and as experimental therapeutic agents (Fig. 2) [3, 4]. Caffeine and other naturally occurring alkylxanthines act as nonselective competitive antagonists of adenosine with respect to at least three of the four subtypes of the ARs. In fact, antagonism of ARs is the most likely mechanism of action of ingested caffeine. These simple xanthine antagonists are of micromolar affinity, but these lead molecules have been optimized for affinity and selectivity. Library screening has pointed the way for the identification of a variety of chemically novel lead structures that have been optimized by medicinal chemists for affinity and selectivity as adenosine antagonists.

Sources of Adenosine, its Transport Mechanisms, and Metabolism

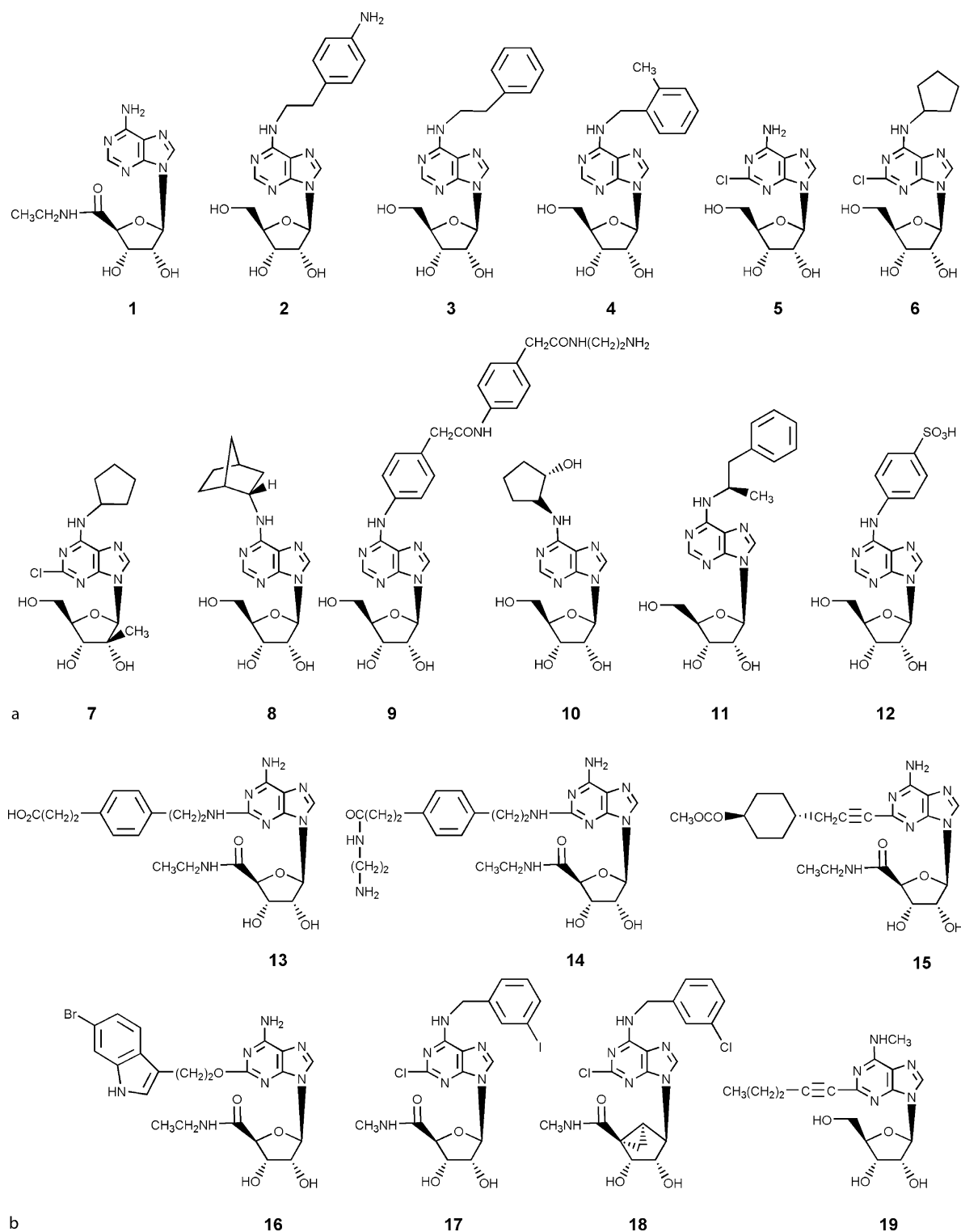
Adenosine production in the synapse is not through vesicular release in response to nerve firing, as is the case for classical neurotransmitters. Rather, adenosine acts as a local autacoid, the release of which increases upon stress to an organ or tissue. Most cells in culture and in situ produce and release adenosine extracellularly. This

endogenous adenosine released tends to influence the outcome of pharmacological studies and can cause misleading results if not properly controlled. Depending on stress factors present, the levels of extracellular adenosine in a given tissue or organ may vary widely, leading to highly variable basal levels of stimulation of the ARs by endogenous adenosine. The level of adenosine may be as low as ~20 nM in the resting brain and as high as 100 µM in severe ischemic conditions. The half-life of adenosine in the blood is short (~1 s), and the peripheral administration of adenosine has no effect on the extracellular concentration of adenosine in the brain. Both neuronal and glial cell function are regulated by adenosine.

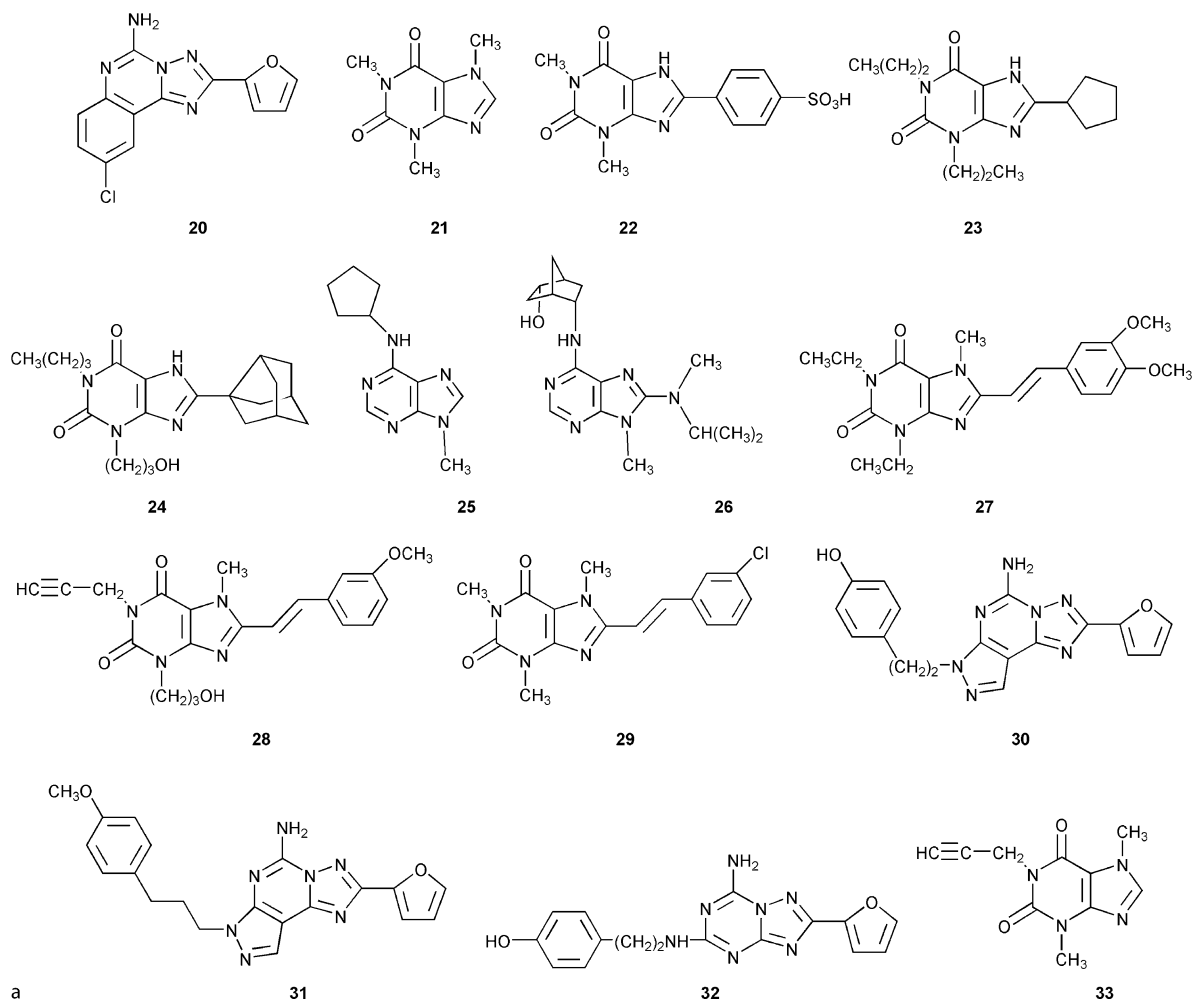
One source of extracellular adenosine may be both from inside the cell, where it is present in millimolar concentrations. As a hydrophilic small molecule, adenosine does not diffuse freely through the intact plasma membrane; rather, it may pass through an equilibrative transporter such as the ENT1 nucleoside transporter, for which there are well characterized inhibitors [7]. Levels of extracellular adenosine may also rise as a result of the enzymatic hydrolysis of extracellular adenine nucleotides or cell lysis. Nucleotide precursors of adenosine, notably ATP and ADP, have their own extracellular signaling properties that are mediated by P₂ receptors. ►Ectonucleotidases, which are also ubiquitously expressed on the cell surface, but with characteristic distribution patterns, cleave adenine nucleoside 5'-phosphate derivatives (including activators of P₂X and P₂Y nucleotide receptors) to eventually form adenosine. There are many classes of ectonucleotidases; however, the most relevant species in the family of ectonucleoside triphosphate dihydrolases (E-NTPDases) that act to breakdown P₂ agonists are apyrase or NTPDase1 (which converts ATP and ADP to AMP) and NTPDase2 (which converts ATP to ADP). A separate enzyme, ecto-5'-nucleotidase (CD73), converts AMP to adenosine. CD73 is characteristically found on the surface of astrocytes but not neurons.

Unlike classical neurotransmitters, adenosine does not have a rapid synaptic uptake system (as for the biogenic amines), and its chemical inactivation system is not as rapid as for the transmitter acetylcholine, for example. Adenosine may be metabolized extracellularly and inactivated with respect to the ARs in a more general fashion by the widespread enzymes adenosine kinase (AK, to produce AMP) and adenosine deaminase (AD, to produce inosine). Both AMP and inosine are only weakly active at ARs, depending on the subtype.

Inhibition of the metabolism of extracellular adenosine or its uptake proteins is being explored for therapeutic purposes. AK inhibitors have been proposed for the treatment of pain and seizures; however, the promising clinical development of these efficacious compounds was discontinued due to toxicity.



Adenosine Receptors. Figure 1 Structures of widely used AR agonists, both nonselective and selective. Affinities/potencies at the ARs are found in [Table 2](#). (a) Nucleoside derivatives that are either nonselective or selective for A_1 receptors (1–12). (b) Nucleoside derivatives that are selective for A_{2A} , A_{2A}/A_{2B} (mixed), or A_3 receptors (13–19).



Adenosine Receptors. Figure 2 Structures of widely used AR agonists, both nonselective and selective. Affinities/potencies at the ARs are found in Table 2. (a) Nonnucleoside derivatives that are either nonselective or selective for A₁ or A_{2A} receptors. (b) Nonnucleoside derivatives that are selective for A_{2B} or A₃ receptors.

Receptor Structure, Signaling Pathways Coupled to ARs, and AR Regulation

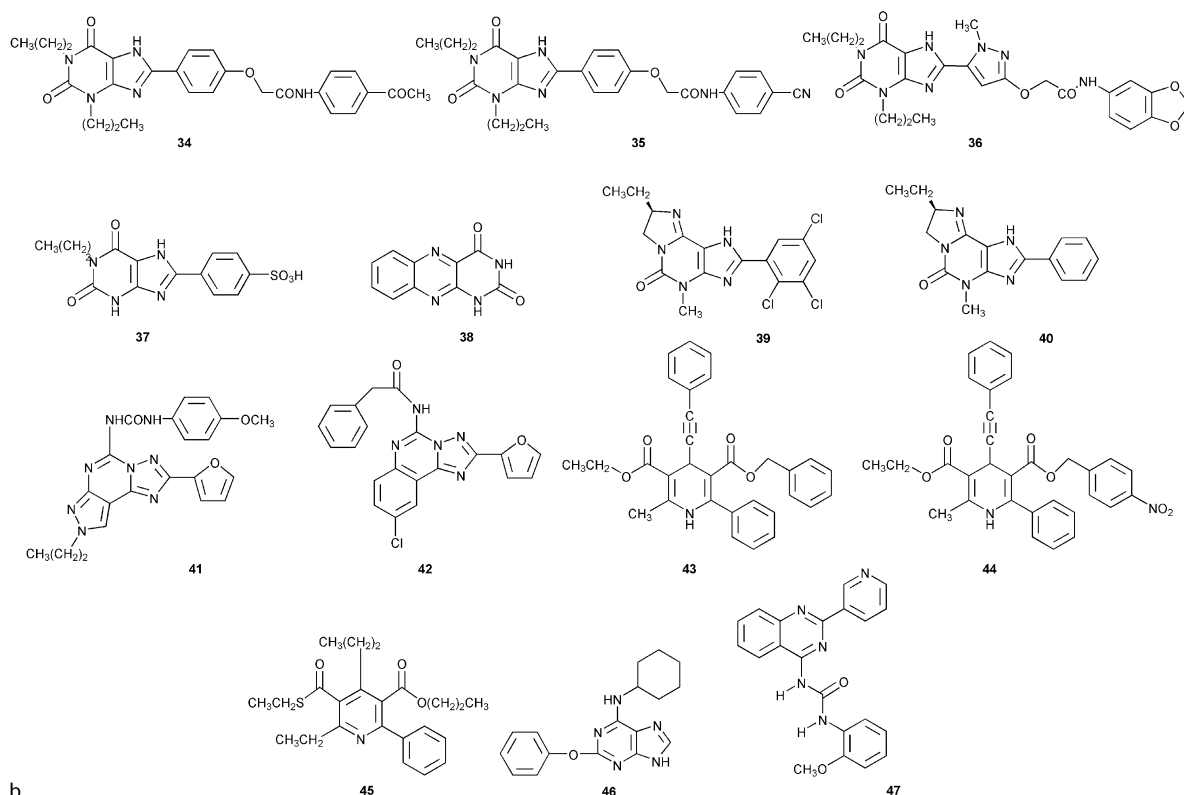
The four mammalian ARs are members of the rhodopsin-like Class A family of **GPCRs**, which contain seven transmembrane helical domains (**TMs**). Characteristics of the four subtypes of the human ARs, length of their primary sequences, their chromosomal localization, and their signaling pathways are given in Table 1. The A_{2A} receptor is considerably longer than the other three subtypes, due to its extended carboxy-terminal.

Two AR subtypes, A₁ and A₃, couple through G_i to inhibit adenylate cyclase, while the other two subtypes, A_{2A} and A_{2B}, stimulate adenylate cyclase through G_s or G_{olf} (for A_{2A}). The A_{2B}AR is also coupled to the activation of PLC through G_q. Furthermore, each of these receptors may couple through the β,γ subunits of the G proteins to other effector systems, including ion channels and phospholipases. Levels of intracellular

calcium increase upon stimulation of ARs, which interact with other second messenger systems. ARs have been found to couple to mitogen-activated protein kinases (MAPKs) in a variety of circumstances, leading to effects on differentiation, proliferation, and cell death.

Crosstalk occurs between ARs and other receptors. For example, an otherwise subthreshold concentration of acetylcholine, as might be present in the Alzheimer's brain, still produces a strong calcium signal when the A₁ AR is costimulated. Crosstalk occurs with the striatal dopamine receptor system, in which a direct physical association (dimerization) occurs between A_{2A} and D₂ receptors, and between other subtypes. The A₁AR forms functional heterodimers with the P2Y₁ nucleotide receptor, which may be stimulated by P2Y₁ receptor agonists but is not blocked by P2Y₁ receptor antagonists.

Downregulation of ARs should be considered in pharmacological studies and in the development of



Adenosine Receptors. Figure 2 (Continued)

agonists for therapeutic purposes. Responses of all four subtypes have been found to desensitize, and down-regulated the receptors. The most rapid downregulation among the AR subtypes is generally seen with the A_{3A} AR.

Ligands and Mechanisms Involved in Ligand Binding

The structure activity relationships (►SAR) of newly synthesized analogues of nucleosides, xanthine heterocycles, and nonxanthine heterocycles have been explored at the ARs. Potent and selective AR antagonists have been prepared for all four subtypes [3, 4], and selective agonists are known for three subtypes [1]. Thus, numerous pharmacological tools are available for in vitro and in vivo use (Table 2). Potent and selective A_{2B} AR agonists are yet to be reported, although several research groups have identified lead compounds.

Agonists: Medicinal chemists have extensively explored the ►SAR of adenosine derivatives as agonists of the ARs. Until recently, with the synthesis of atypical adenosine agonists that are pyridine-3,5-dicarbonitrile derivatives, nearly all AR agonists have been purine nucleoside derivatives. In general, for the adenine moiety of adenosine, modifications at the N^6 position have led to selectivity for the A_1 AR, and modifications at the 2 position, especially with ethers, secondary amines, and alkynes, have led to selectivity for the A_{2A} AR. Commonly used A_1 AR agonists that are N^6 -cycloalkyl

derivatives are the 2-chloro analogue CCPA **6** and S(-)-ENBA **8**, which are more highly selective than R-PIA **11**. When using the human ARs, S(-)-ENBA is more highly A_1 AR-selective than CCPA or its 2-H analogue CPA. Substitution of the 2' position of the ribose moiety of CCPA with a methyl group results in **7**, with a high selectivity for the A_1 AR. The hydroxy derivative **10** of CPA is a clinical candidate selective for the A_1 AR. The A_1 AR agonist SPA **12** contains a sulfonate group, which tends to prevent passage across the blood–brain barrier. Some N^6 derivatives are not selective for the A_1 AR; for example, the arylamino derivative APNEA **2**, which is an analogue of **3**, and metrifudil **4**, are relatively nonselective. 2-Chloroadenosine **5** is a non-selective agonist that is subject to cellular uptake and nonreceptor-mediated effects.

The substitution at the 2 position may also lead to A_{2A} AR enhancing effects, which are further boosted with an uronamido substitution at the 5' position of adenosine. When the 5'-*N*-alkyluronamide group alone is present, high affinity [at the A_{2A} AR but not selectivity] is typically observed, similar to the nonselective agonist NECA **1**. By combination of this-uronamide group with the appropriate 2 position substitution, selectivity may be achieved for the A_{2A} AR. For example, the 5'-*N*-ethyl derivatives CGS21680 **13** and ATL-146e **15** are both selective in

Adenosine Receptors. Table 1 Characteristics of the four subtypes of adenosine receptors (human, unless noted)

Receptor subtype	A ₁	A _{2A}	A _{2B}	A ₃
Genbank accession number	S45235	S46950	X68487	L22607
Amino acids	326	412	332	318
Theoretical molecular model (PBD ID)	–	1UPE	–	1OEA; 1R7N
Chromosomal localization	1q32.1	22q11.2	17p11.2–12	1p13.3
G protein coupling selectivity	G _{i/o}	G _s ; G _{olf}	G _s ; G _q	G _{i/o}
Key residues for ligand recognition	H278 (7.43)	E151, E169 (EL2); F182 (5.43); H250 (6.52); N253 (6.55); H278 (7.43); S281 (7.46)	F59 (2.56)	H95 (3.37); N250 (6.55); H272 (7.43)
Mutations specific for reduced agonist potency	E16A (1.39); C85S (3.30); T277A (7.42); L65T (2.60) (rat); I69S (2.64) (rat)	T88A (3.36); S277A (7.42); H278Y (7.43)	–	
Mutations specific for reduced antagonist potency	H251L (6.52) (bovine)	V84L (3.32)	–	K152A (EL2); W243A (6.48)

The A₁AR is found in the brain (cortex, cerebellum, hippocampus), dorsal horn of the spinal cord, eye, adrenal gland, heart (atrium), skeletal muscle, liver, kidney, adipose tissue, salivary glands, esophagus, colon, antrum, and testis. The A_{2A}AR is found in spleen, thymus, leukocytes (both lymphocytes and granulocytes), and blood platelets. In the brain it is restricted to the striatum, nucleus accumbens, olfactory tubercle, and large striatal cholinergic interneurons. The message for A_{2B} and A₃ARs occurs throughout the CNS and in many peripheral tissues.

binding to the rat A_{2A}AR, but are less selective at the human subtypes. CGS21680 crosses the blood–brain barrier to only a small degree. The aminoethyl derivative APEC **14**, however, crosses the blood–brain barrier and is more potent than CGS21680 in causing locomotor depression through a central mechanism.

There are not yet any agonists that are truly selective for the A_{2B}AR, known as the “low affinity” adenosine A₂ receptor. A novel agonist, the 2-(6-bromotryptophol) ether derivative MRS3997 **16**, is a full agonist with mixed selectivity at A_{2A} and A_{2B}ARs.

The A₃ agonists CI-IB-MECA **17** and its corresponding 2-H analogue IB-MECA are widely used as selective agonists of the A₃AR, although even more selective agents are now known, including those in which the ribose-like ring has been conformationally locked in the receptor-preferring north conformation, such as MRS3558 **18**. HEMADO **19** is a human A₃AR-selective agonist, but its selectivity does not generalize across species.

Antagonists: The classical AR **▶antagonists** are **▶xanthines** derivatives such as caffeine **21** and theophylline (1,3-dimethylxanthine). The micromolar affinity of the naturally occurring antagonists has been greatly exceeded with the introduction of selective antagonists, even reaching subnanomolar affinity. For example, the A₁AR-selective antagonists **23** and **24** are xanthine derivatives. The 8-cyclopentyl derivative DPCPX **23** is highly A₁AR selective in the rat and less A₁AR selective among the human AR subtypes. DPCPX is an **▶inverse**

agonist at the A₁AR, while the adenine derivative N-0840 **25** is a **▶neutral antagonist**. In general, modifications of the xanthine scaffold at the 8 position with aryl or cycloalkyl groups have led to selectivity for the A₁AR, although the water soluble 8-sulfophenyl derivative **22** is nonselective. Persistent problems in the use of xanthine derivatives as AR antagonists of the A₁AR are their low aqueous solubility and their interaction at the A_{2B}AR. Use of adenine derivatives, such as the inverse agonist WRC-0571 **26**, provides A₁AR-selective antagonists that have low affinity at the A_{2B}AR.

Modifications of **▶xanthines** at the 8 position with alkenes (specifically styryl groups) have led to selectivity for the A_{2A}AR. The 8-styrylxanthine derivatives KW6002 **27**, MSX-2 **28**, and CSC **29** are moderately potent A_{2A}AR antagonists [3]. Some 8-styrylxanthine derivatives, especially CSC, have been discovered to inhibit monoamine oxidase-B as well. High selectivity of **▶xanthines** at the A₁, A_{2A}, and A_{2B} (e.g., MRS1754 **35** and its *p*-COCH₃ analogue MRS1706) ARs has been achieved. **▶Xanthines** that are selective for the A₃AR remain to be designed, however, PSB-II **40** contains an elaborated xanthine ring system.

In recent years, an enormous diversity of heterocyclic structures has been reported as AR antagonists (Table 2) [2]. For example, the nonselective triazoloquinoline antagonist CGS15943 **20**, first introduced in the early 1990s, has given rise to numerous derivative

Adenosine Receptors. Table 2 Affinity of commonly used adenosine receptor agonists and antagonists for defining pharmacologically adenosine receptor subtypes

		K _i (nM), unless noted			
Selectivity	Compound	A ₁ AR ^a	A _{2A} AR ^a	A _{2B} AR ^a	A ₃ AR ^a
	Agonists				
None	1 NECA	14	20	140 ^c	25
	2 APNEA	14 ^b	172 ^b	n.d.	116 ^b
	3 PhEt-Ado	12.9	676	67,000 ^c	2.1
	4 Metrifudil	59.6 ^b	24.1 ^b	>100,000 ^c	47.2
	5 CADO	7.5	630	24,000 ^c	87
A ₁	6 CCPA	0.83	2270	18,800 ^c	38
	7 2'-MeCCPA	3.3	9580	37,600	1150
	8 S-ENBA	0.38	>10,000	>10,000 ^c	915
	9 ADAC	0.85 ^b	210 ^b	n.d.	13.3
	10 GR79236	3.1 ^b	1300 ^b	n.d.	n.d.
	11 R-PIA	2.0	860	58,000 ^c	8.7
	12 SPA	70 ^b		52,000 ^c	
A _{2A}	13 CGS21680	289	27	>10,000 ^c	67
	14 APEC	400 ^b	5.7 ^b	50 ^b	
	15 ATL-146e	77	0.5	n.d.	45
A _{2B}	16 MRS3997		128	150	
A ₃	17 Cl-IB-MECA	220	5360	>100,000 ^d	1.4
	18 MRS3558	260	2300	>10,000 ^d	0.29
	19 HEMADO	327	1230	>10,000	1.1
	Antagonists				
None	20 CGS15943	3.5	4.2	16	51
	21 Caffeine	29,000	48,000	10,400	13,300
	22 SPT	4020	7050	1330	5890
A ₁	23 DPCPX	3.9	129	56	3,980
	24 PSB36	0.12 ^b	552 ^b	187	2300
	25 N-0840	750			
	26 WRC-0571	1.7	105	n.d.	7940
A _{2A}	27 KW6002	2830	36	1800	>3,000
	28 CSC	28,000 ^b	54 ^b	n.d.	n.d.
	29 MSX-2	2500	8	>10,000	>10,000
	30 SCH58261	725	5.0	1110	1200
	31 SCH442416	1110	0.048	>10,000	>10,000
	32 ZM241,385	774	1.6	75	743
	33 DMPX		11,000		
A _{2B}	34 MRS1706	157	112	1.39	230
	35 MRS1754	403	503	2.0	570
	36 MRE 2029-F20	245	>1000	3.0	>1000
	37 PSB1115	>10,000	24,000 ^b	53.4	>10,000
	38 Alloxazine			2400	

compounds, including A_{2A} AR-selective antagonists (**30**, and **31** and the triazolotriazine **32**), A_{2B} AR-selective antagonists (**34–37**), and A_3 AR-selective antagonists (**41** and **42**). The triazolotriazine – ZM241,385 **32** – and the pyrazolotriazolopyrimidines

– SCH-58261 **30** and SCH442,416 **31** – are highly potent and selective A_{2A} AR antagonists [3]. SCH442,416 displays > 23,000-fold selectivity for the human A_{2A} AR (K_i 0.048 nM) in comparison to human A_1 AR and IC_{50} > 10 μ M at the A_{2B} and A_3 ARs.

Adenosine Receptors. Table 2 Affinity of commonly used adenosine receptor agonists and antagonists for defining pharmacologically adenosine receptor subtypes (Continued)

Selectivity	Compound	K _i (nM), unless noted			
		A ₁ AR ^a	A _{2A} AR ^a	A _{2B} AR ^a	A ₃ AR ^a
A ₃	39 PSB-10				0.43
	40 PSB-11	1640	1280	2100 ^d	3.5
	41 MRE 3008-F20	1200	141	2100	0.82
	42 MRS1220	305 ^b	52.0 ^b	n.d.	0.65
	43 MRS1191	>10,000	>10,000	>10,000 ^c	31.4
	44 MRS1334	>100,000	>100,000	n.d.	2.7
	45 MRS1523	15,600 ^b	2050 ^b	n.d.	18.9
	46 MRS3777	>10,000	>10,000	>10,000 ^c	47
	47 VUF5574	>10,000	>10,000		4

n.d., not determined.

^aAffinity at human A₁, A_{2A}, A_{2B}, and A₃ARs, unless noted, expressed as K_i (nM).^bAffinity determined at rat ARs.^cPotency in a cyclic AMP functional assay.

Alloxazine **38** is a weak, nonxanthine antagonist that is slightly selective for the A_{2B}AR.

Although the simple xanthine antagonists have not provided suitable analogues with A₃AR selectivity, a cyclization of the xanthine nucleus, leading to imidazopurinones **39**, **40**, and their congeners has. For A₃AR, most of the successful leads have come from chemically diverse heterocycles. The dihydropyridine derivatives MRS1191 **43**, its nitro analogue MRS1334 **44b**, and the pyridylquinazoline derivative VUF5574 **47** are potent, selective A₃AR antagonists in the human, but are weak at the rat A₃AR. Nevertheless, MRS1191 has been used successfully in murine species. MRS1220 **42** is very potent and selective at the human A₃AR but not at the rat or the mouse receptor. There is a marked species dependence of antagonist affinity at the A₃AR. Commonly used A₃AR antagonists must be treated with caution in non-human species other than human. In general, one must be cognizant of potential species differences for both AR agonists and antagonists. The pyridine derivative MRS1523 **45** is a moderately selective A₃AR antagonist units for both the rat and human.

Variations in the relative efficacy of nucleosides, depending on structure, have been noted. This is especially pronounced for the A₃AR, at which changes on the adenine moiety (*N*⁶ and 2 positions) and ribose moiety can either reduce efficacy to the point of pure antagonism (i.e., combination of 2-Cl and *N*⁶-(3-iodobenzyl)) or guarantee a robust, nearly full activation of the A₃AR (i.e., 5'-uronamide) [2]. Such nucleoside-derived A₃AR antagonists tend to have selectivity that is more general across species.

Radioligands: Radioligands commonly used for the ARs are: A₁ agonist [³H]CCPA **6**, antagonist [³H]

DPCPX **23**; A_{2A} agonist [³H]CGS21680 **13**, antagonist [³H]ZM241,385 **32** or [³H]SCH58261 **31**; A₃ agonist [¹²⁵I]I-AB-MECA (the *N*⁶-(4-amino-3-iodobenzyl) 2-H analogue of **17**) and antagonist [³H]PSB-11 **40**. Ligands for in vivo positron emission tomographic imaging of A₁ and A_{2A}ARs have been developed [3]. Potent fluorescent ligands have been reported for A₁ and A_{2A}ARs.

Allosteric modulation: In addition to AR agonists and antagonists that interact directly with the primary (orthosteric) site of the receptor, ►allosteric modulators of agonist action are also under consideration for disease treatment. Such modulators, either positive enhancers or negative allosteric inhibitors might have advantages over the directly-acting (orthosteric) receptor ligands. The action of the allosteric compounds would depend on the presence of a high local concentration of adenosine, which often occurs in response to a pathological condition [4]. In some cases (dependent on tissue, receptor subtype, and other conditions), one would wish to boost the adenosine effect, and therefore, an allosteric enhancer would be useful. In other cases, the elevated adenosine may be detrimental, in which instance one would want to apply a negative modulator. ►Allosteric modulators have been explored and are under development for the A₁ and A₃ AR subtypes [4].

Receptor modeling: Each of the ARs has been modeled based on homology to bovine rhodopsin (the only GPCR with an available crystal structure) or by other methods. Several such theoretical models are now available (Table 1). Molecular modeling of the ARs and ►ligand docking have provided insights into the putative binding sites of all of the subtypes, which has aided in ligand design [1, 3]. Essential residues for the binding of ligand and activation of the receptors have

TM1		*	TM2	*	TM3		
A1		MP---	PSISAFQAAVIGIEVLIALVSVPGNVLIWAVKVNQALRDATFCFIVSLVADVAVGALVPLAILINIGPQTYFHTCLMVACPVLIILT			91	
A2A		MP----	IMGSSVYITVELATAVLAILGNVLVCAVWLNLSNLQNVNTYFVVS	LAAADIAVGVLAI	PPFAITISTGFCAACHGCLFIACFVLVLT	88	
A2B		M----	LLETQDALYVALELVIAALS	VAGNVLVCAAVGTANTLQTP	TPTNYFLVSLAADVAVGLFAIPFAITISLGFCTDFYGCFLFACFVLVLT	89	
A3		MPNNSTALSLANVTYITMEIF	IGLCAIVGNVLVICVVKLNPSLQTTTFYFIVSLALADIAVGVLVMP	LAIIVVSLGITIHFYSCLFMTCLLLIFT		94	
		*	TM4	*			
A1	92	QSSILALLAIAVDRLRVKIP	PLRYKMVVTPRRAVAIA	GCWILSFVVGLTPMFGWN	--N-LSAVERAW-AA-N-GSMGEPVIK-C----	E- 170	
A2A	89	QSSIFSLLAIAIDRYIAIRI	PLRYNGLVTGTRAKGII	AICWVLSFAIGLTPMLGWN	-----N--CGQP-KEG-K-N-HSQCGEGQVAC	166	
A2B	90	QSSIFSLLAIVADRYLAICV	PLRYKSLVTGTRARGVIA	VLWVLAFGIGLTPPLGWN	SKDSATN-N--CTEPWD-G-T-T-NES-CC--LVKC	168	
A3	95	HASIMSLLAIAVDRLRVKLT	VRYKRVTTTHRIWLALG	LCWLVSEFLVGLTPMFGWN	-MK-LTS-E--Y-H--R-N-V-T-FLS-C-----Q-	167	
		TM5	*	TM6	*		
A1	171	-FEKVISMVYVFNFFVWVLP	LLMLVLIYLEVYLRKQ	LNKKVSASSG--DPQKYYG	KELKIAKSLALILFLFALS	WLPLHILNCITLF 259	
A2A	167	LFEDVVPNNYVYFNF	FACVLVPLLLMLGVYLR	IPLAARRQLKQMESQPL	PGERARSTLQKEVHAAKSL	AIIVGLFALCWLPLHIINCPTFF 258	
A2B	169	LFENVVPMSYMVYFNF	FGCVLPPLLIMLVYIKI	FLVACRQLQRTLM----	DHSRTTLQREIHAAKSL	AMIVGIFALCWLPHAVNCVTLF 259	
A3	168	-FVSVMRDMYVYFSE	FLTWIFPLVVMCAIYLD	IFYYIRNKLNLNSNSK---	ETGAFYGRFETAKSL	FLVLFLFALS	WLPLSIINCIIYF 255
		TM7	*				
A1	260	CPSC--HKPSILTYIAIFL	THGNSAMNP	IVYAFRIQKFRVT	FLKIWNDFRCQPAPPIDED	LPEERPD----- 326	
A2A	259	CPDCS-HAPLWMLYLAIVL	SHNSVNNPFIYAYR	IREFRQTRFKIIRSHVLR	QQEPPKAAGTSARVLA	HAHGS	DGEQVSL 336
A2B	260	QPAQGNKPKWAMNMAIL	SHANSVNNPFIYAYR	NRDPFRTFHKIISRYLL	CQAD-VKSGNGQAGVQ	PALG-----VGL 332	
A3	256	NG---EVEQLVLYMGILL	SHANSMMNP	IVYAYKIKKFKET	YLLILKACVVCHPS	SLDTSIEKNSE----- 318	

Adenosine Receptors. Figure 3 An alignment of the primary sequences of the four human AR subtypes. Regions of conservation are highlighted. * indicates the most conserved (X.50) residue in each TM region. Bold residues correspond to those indicated in Table 1. The A2A receptor is truncated in the carboxy-terminal region.

been defined through both modeling and site-directed mutagenesis. Similar to other GPCRs having small molecular ligands, ►TMs 3, 5, 6, and 7 of the ARs are thought to be most closely associated with bound agonists and antagonists. An alignment of the primary sequences of the four human AR subtypes (Fig. 3) indicates a high degree of homology within the ►TMs. The second extracellular loop (EL2) is highly variable in sequence and contains a relatively high content of charged residues, in addition to the connection point (Cys residue) of a conserved disulfide bridge.

Key functional residues for ligand binding and activation that have been identified are listed in Table 1 (also in bold in 3). Extensive mutagenesis of the ARs has helped in defining a putative, hydrophilic ribose-binding region spanning ►TMs 3 and 7. The putative adenine-binding region contains more hydrophobic residues, particularly in the vicinity of the N⁶ substituent. The EL2 also is thought to coordinate the ligands.

The binding and activation steps of receptor action have been dissected computationally, although not yet in a global fashion. The conformational dynamics of the activation of the A₃AR have been approximated with respect to isolated portions of the receptor.

Drugs

Presently, only adenosine itself is approved for clinical use. It is used widely in the treatment of supraventricular tachycardia and in cardiac stress imaging to assess coronary artery disease [5]. Other agonists and antagonists and an ►allosteric modulator of the A₁ receptor are in clinical trials for a variety of indications.

Selective AR agonists are undergoing clinical trials for cardiac arrhythmias and pain (A₁); cardiac imaging and inflammation (A_{2A}); colon cancer, rheumatoid arthritis, psoriasis, and dry eye (A₃). Selective AR antagonists are either in or advancing toward clinical trials for kidney disorders (A₁); Parkinson's disease (A_{2A}); diabetes and asthma (A_{2B}); cancer and glaucoma (A₃).

►Purinergic System

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Adenoviruses

►Gene-Therapy Vectors

Adenylate Cyclase

► Adenylyl Cyclases

Adenylyl Cyclases

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Synonyms

Adenylyl cyclase (preferred); Adenylate cyclase;
Adenyl cyclase (original); ATP:pyrophosphate lyase;
Cyclizing (E.C.4.6.1.1.)

Definition

Adenosine 3':5'-monophosphate (►cAMP) regulates effects in all eukaryotic cells, principally through activation of cAMP-dependent protein kinase (►PKA), but also through cAMP-gated ion channels (►CNGs) and select guanine nucleotide exchange factors (►Epacs) (Fig. 1). Cellular levels of cAMP levels reflect the balance of activities of adenylyl cyclases (AdCy: $5'\text{-ATP} \rightarrow \text{cAMP} + \text{PP}_i$) and cAMP ►phosphodiesterases (PDE: $\text{cAMP} \rightarrow 5'\text{-AMP}$). Adenylyl cyclases occur throughout the animal kingdom and play diverse roles in cell regulation [1].

Basic Characteristics

Classes of Adenylyl Cyclases

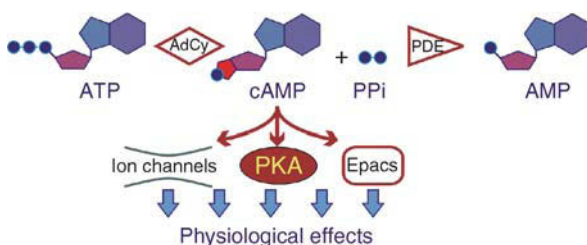
Adenylyl cyclases belong to the larger class of purine nucleotide cyclases. These have been divided into classes I–VI [2]. Class I cyclases include those in gram negative bacteria, e.g. *Escherichia coli* and *Yersinia pestis*, in which cAMP levels respond to external

nutrient levels and mediate effects on transcription factors and gene expression. Class II cyclases comprise the extracellular soluble toxins of certain pathogens (see below under “*Bacterial and other adenylyl cyclases*”), e.g. those of *Bacillus anthracis*, *Bordetella pertussis*, and *Pseudomonas aeruginosa*. Class III cyclases (with subclasses IIIa–IIIc) include most adenylyl and guanylyl cyclases (Fig. 2). These enzymes respond to changes in the extracellular environment. In mammals this occurs via hormones, neurotransmitters, odorants, or tastes. In lower organisms influences may be via changes in ionic factors, glucose, bicarbonate, or serum factors, and through osmoregulation, chemotaxis, phototaxis, or pH, in various bacteria. Class IV–VI cyclases are tentative assignments with few members each, but contain soluble and smallest adenylyl cyclases (~180 amino acids). The Class IV adenylyl cyclase of *Y. pestis* has been crystallized and its structure determined. The enzyme in *Prevotella ruminicola* was designated as a Class V cyclase [2]. And the enzyme found in the nitrogen-fixing bacterium *Rhizobium etli* was designated as a Class VI adenylyl cyclase.

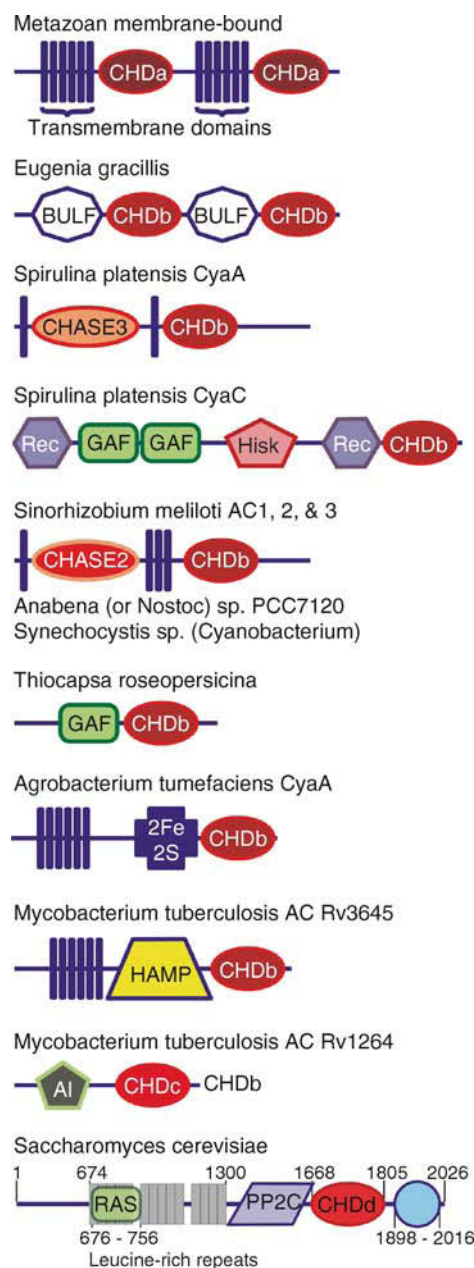
Mammalian Adenylyl Cyclases

Of the ten known ►isozymes of mammalian adenylyl cyclase (AC1–AC10; Table 1) all but one are membrane-bound and are regulated via cell-surface receptors linked to heterotrimeric ($\alpha\beta\gamma$) stimulatory (G_s) and inhibitory (G_i) guanine nucleotide-dependent regulatory proteins (►G-proteins) (Fig. 3, Table 2) [1]. These receptors are referred to as G-protein coupled receptors, or GPCRs, and mediate effects of stimulatory and inhibitory hormones and neurotransmitters. G_{α_s} stimulates all of these isozymes save the soluble AC10, and the plant-derived diterpene, ►forskolin, stimulates all isozymes, save AC9 and AC10, by binding within the cleft formed by the enzyme's two cytosolic domains ($C_1 \cdot C_2$; see below). The several isozymes differ more significantly in their responses to G_{α_i} and $G_{\beta\gamma}$ and in the physiological responses they control (Table 2). For example, G_{α_i} inhibits some but not all isozymes and $G_{\beta\gamma}$ inhibits AC1 and AC8, but significantly stimulates AC2, AC4, and AC7, and has reportedly different effects on AC5 and AC6 (Table 2).

Stimulation and inhibition of the enzyme by the GPCR-G-protein cycle occur by analogous mechanisms. Agonists induce hormone receptors to increase a $G_{\alpha}\text{-GDP-GTP}$ exchange and subsequent $G_{\alpha\beta\gamma}$ dissociation ($\text{GDP} \cdot \alpha_x\beta\gamma + \text{GTP} \rightarrow \text{GTP} \cdot \alpha_x + \beta\gamma + \text{GDP}$) (Fig. 4). Consequently, agents that affect either the dissociation of either G_i or G_s , or the association of their respective α_s , α_i , or $\beta\gamma$ subunits with adenylyl cyclase could affect rates of cAMP formation in enzyme preparations or in intact cells and tissues. There are several important examples. G_{α_s} is stably activated by poorly hydrolyzable analogs of GTP, e.g. GTP γ S



Adenylyl Cyclases. Figure 1 Synthesis, degradation, and actions of cAMP.



Adenylyl Cyclases. Figure 2 Domain organization of several class III adenylyl cyclases. Domains are abbreviated: CHD: cyclase homology domains for subclasses (IIIa – IIIc), predicted transmembrane domains are indicated as vertical blue bars, BLUF: blue light receptor with tightly bound flavin, CHASE: (Cyclase/histidine kinase-associated sensing extracellular) domain, GAF switch domain: cGMP-binding phosphodiesterases, Adenylyl cyclases, and *E. coli* transcription factor FhlA, Rec: receiver domain; HisK: histidine kinase domain, 2Fe-2S: two iron-two sulfur cluster domain, HAMP: tandem amphipathic α -helices present in Histidine kinases, Adenylyl cyclases, Methyl-accepting chemotaxis proteins, and Phosphatases, AI: auto-inhibitory domain, leucine-rich

or GPP(NH)P, and its activation is hindered by GDP β S. A less obvious example is **fluoride**. It activates most mammalian adenylyl cyclases, indirectly through its AlF_4^- complex with $\text{G}\alpha_s \cdot \text{GDP}$. Another example includes the ADP-ribosyltransferase activities of bacterial toxins. The toxin of *Vibrio cholerae* catalyzes the ADP-ribosylation from NAD of $\text{GTP} \cdot \text{G}\alpha_s$, and that of *Bordetella pertussis* similarly ADP-ribosylates α_i of $\text{GDP} \cdot \alpha_i \beta \gamma$, preventing its dissociation. In both cases the effect is elevated adenylyl cyclase activity and contributes to the pathophysiology of these bacteria. Of therapeutic relevance, of course, are agents acting as agonist or antagonist on GPCRs coupled to adenylyl cyclase, with the prominent example being antagonists of β -adrenergic receptors (i.e. β -blockers).

Activities of all isozymes are affected by Ca^{2+} . At higher concentrations (mM) Ca^{2+} is inhibitory through competition with divalent cation required for catalysis (see below). At lower concentrations ($< \mu\text{M}$), Ca^{2+} regulates activity physiologically. This can be through: (i) a direct effect at the catalytic active site, increasing activity of AC10, or decreasing activity of AC5 and AC6; (ii) as a Ca^{2+} /calmodulin complex, activating ACs 1,3,8; (iii) with calcineurin to inhibit AC9; or (iii) indirectly through activation of PKC. Phosphorylation of adenylyl cyclase varies among the isozymes and is determined by differences in their primary sequences and is catalyzed by cAMP-dependent protein kinase (PKA) or protein kinase C (PKC) (**Protein Kinase C**) (Table 2). Activity of adenylyl cyclases can be indirectly influenced by the specific phosphorylation of hormone receptors or of **G-proteins**.

Membrane-bound forms of mammalian adenylyl cyclases exhibit a putative topology with twelve membrane-spanning regions and two largely homologous ~ 40 kDa cytosolic domains (C_1 and C_2) (cf. Fig. 2). Differences in N-terminal and other domains are significant and influence regulation by a variety of agents as noted above (cf. Table 2). AC5(C_1) and AC2(C_2) domains have been separately expressed, recombined, and the resulting structure was solved in complex with $\text{GTP} \cdot \text{G}\alpha_s$ (Fig. 5) [3]. $\alpha_s \cdot \text{GTP}$ activates the enzyme through interaction with C_2 , yielding the active enzyme: Inhibition of adenylyl cyclase may occur by interaction of $\text{G}\alpha_i$ with the C_1 domain of adenylyl cyclase, yielding $\text{GTP} \cdot \alpha_i \cdot C$, or by the recombination of $\beta \gamma$ with $\text{G}\alpha_s$. The structure obtained with $\beta\text{-L-2',3'-dd-5'-ATP}$ allowed the demonstration that the pseudo symmetric cleft formed by the $C_1 \cdot C_2$ domains binds 5'-ATP, forskolin, and cation at

repeats are indicated a vertical grey bars, PP2C: protein phosphatase type 2C catalytic domain, RAS, RAS-associated domain, and CAP: cyclase activating protein. This figure was modified from [2].

Adenylyl Cyclases. Table 1 Source, accession numbers, size, and gene loci for mammalian adenylyl cyclases

ADCY	Source	Reference accession numbers	Base pairs (mRNA)	Amino acids	Respective species chromosome
1	Bovine	NM_174229	3978	1134	4
	Human	NM_021116	12499	1119	7p13-p12
	Mouse	NM_009622	12259	1118	11 1.25 cM
2	Rat	NM_031007	4008	1090	17p14
	Human	NM_020546	6553	1091	5p15.3
	Mouse	NM_153534	4211	1095	13 41.0 cM
3	Rat	NM_130779	4533	1144	6q14
	Human	NM_004036	4342	1144	2p24-p22
	Mouse	NM_138305	3674	1145	12A-B
4	Rat	NM_019285	3357	1064	15p13
	Human	NM_139247	3320	1077	14q12
	Mouse	NM_080435	3414	1077	14D3
5	Rat	NM_022600	4847	1262	11q22
	Human	NM_183357	3842	1261	3q13.2-3q21
	Mouse	NM_001012765	7069	1262	16B-5
6	Rat	NM_012821	6036	1166	7q36
	Human	NM_015270 (1)	6594	1168	12q12-q13
	Human	NM_020983 (2)	5877	1115	12q12-q13
	Mouse	NM_007405	6038	1168	15F
7	Mouse	NM_007406 (1)	5199	1099	8 40.0 cM
	Mouse	NM_001037723 (2)	4750	1099	8 40.0 cM
	Mouse	NM_001037724 (3)	5938	1099	8 40.0 cM
	Human	NM_001114	6138	1080	16q12-q13
8A	Rat	NM_017142	4601	1248	7q33
	Human	NM_001115	6005	1251	8q24.2
	Mouse	NM_009623	5064	1249	15 37.5 cM
9	Mouse	NM_009624	4457	1353	16 2.0 cM
	Human	NM_001116	7732	1353	16p13.3
10	Human	NM_018417	5061	1610	1q24
Sacy	Mouse	NM_173029	5211	1614	1 H2.3
	Rat	NM_021684	5177	1608	13q23

Notes to Table 1:

(a) Adenylyl cyclases have been numbered in the order in which they were cloned and sequenced. In databases they are referred to as *adcy#*, with the exception of the soluble AC10, which is referred to variably as *Sacy* or *Sac*.

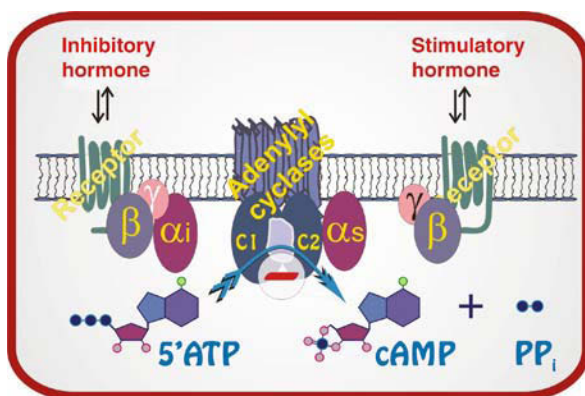
(b) Sources are those for which the database entries are given. The first source listed is the original source from which the isozyme was cloned. In some instances there are variant forms as indicated. For AC8 there are three splice variants; data are provided for variant 8A.

(c) Accession numbers are for the Reference Sequence (*RefSeq*) collection of data from the National Center for Biotechnology Information (NCBI). Values pertinent to the mammalian isozymes of adenylyl cyclases are compiled here. The link is: <http://www.ncbi.nlm.nih.gov/> These numbers link to a "...comprehensive, integrated, nonredundant set of sequences, including genomic DNA, transcript (RNA), and protein products for several major research organisms. *RefSeq* standards serve as the basis for medical, functional, and diversity studies; they provide a stable reference for gene identification and characterization, mutation analysis, expression studies, polymorphism discovery, and comparative analyses. *RefSeqs* are used as a reagent for the functional annotation of some genome sequencing projects, including those of human and mouse."

two sites [4]. The active site shares topology and reaction mechanism with [guanylyl cyclases](#), with which there is considerable homology, and with oligonucleotide polymerases.

Although there is substantial homology among the membrane-bound forms of the mammalian adenylyl cyclases, the striking differences in the character and

extent of regulation by a variety of agents imply that primary and secondary structural characteristics are important determinants in the interactions of the enzyme with cell constituents and hence will regulate enzyme activity, the rate of formation of cAMP, and the downstream effects that this will have. All the studies on mammalian adenylyl cyclases



Adenylyl Cyclases. Figure 3 Membrane localization, topology, and regulation of mammalian adenylyl cyclases.

notwithstanding it is uncertain if all forms and variants of the enzyme have been identified, whether all modes of regulation have been determined, when during development, cell life cycles, and cell–cell interactions that specific isozymes are expressed, and how these processes are regulated. Perhaps because of this, the enzyme family continues to be a focus of much research and even as targets for drug discovery.

Catalytic Mechanism

Catalysis by adenylyl cyclases involves cation-mediated attack of the 3'-OH on the α -phosphate of 5'-ATP, with PP_i as leaving group. It is a reversible bireactant sequential mechanism with free cation and cation •5'-ATP as substrates and cAMP, cation• PP_i , and

Adenylyl Cyclases. Table 2 Regulatory characteristics of mammalian adenylyl cyclases

AdCy	Effect of $G\alpha_i$	Effect of $G\beta\gamma$	Effects of Ca^{2+} and/or calmodulin	Effects of protein kinases	Tissue distribution	Physiological functions
1	↓	↓	↑	No Δ PKA	Brain (neuron), adrenal (medulla)	Neurotransmission, synaptic plasticity, LTP, memory, circadian rhythm
2	↓	↑	No Δ	No Δ PKA ↑ PKC	Brain, lung, skeletal muscle	Synaptic plasticity, arrest of cell proliferation
3	↓	no Δ	↑ (In vitro)		Olfactory epithelium, brain, adrenal, adipose, pancreas	Olfactory response to odorants
4		↑	No Δ		ubiquitous	
5	↓	↑↓	↓ (No CaM)	↓ PKA ↑ PKC α/ζ	Heart, brain (striatum)	Cardiac function, Ca^{2+} -dependent regulation
6	↓	↑↓	↓ (No CaM)	↓ PKA ↓ PKC	Heart, kidney, Brain, liver, widespread	Cardiac function, Ca^{2+} -dependent regulation, hormonal regulation of gluconeogenesis, cell proliferation, coincidence detector for NO
7		↑	No Δ	↑ PKC	Brain, platelets, heart, spleen, lung	Ethanol dependency
8		↓	↑		Brain, lung	Neurotransmission, LTP, synaptic plasticity, memory
9			↓ (Ca^{2+} /calcineurin)	↓ PKC	Skeletal muscle, heart, brain, pancreas	Neurotransmission
10	No Δ	No Δ	↑ (no CaM)		Testes (germ cells), widespread	HCO_3^- sensor; defect associated with absorptive hypercalciuria

Notes for Table 2:

(a) Empty cells imply that no information was available. Effects of additions on adenylyl cyclase activity are as indicated: up (↑) arrow: increase, down (↓) arrow: decrease, or "no Δ " (tested, but no effect on activity seen).

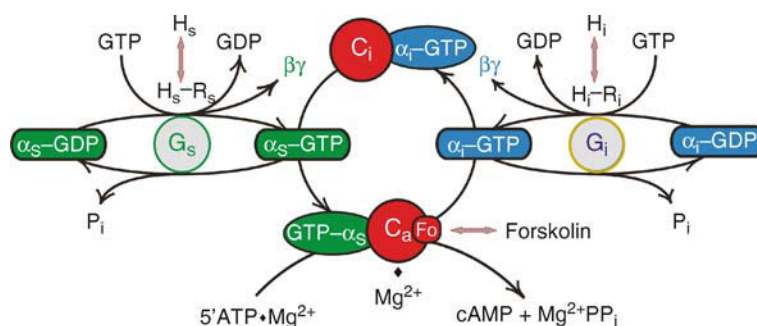
(b) Effects of $G\alpha_i$ or $G\beta\gamma$ are on enzyme stimulated by either $G\alpha_s$ or forskolin. In some instances differences were noted in the effects of different isoforms of β or γ . These are not distinguished here. For AC5 and AC6 both stimulation and inhibition have been reported, the difference being conditionally dependent on stimulation and whether or not full-length enzyme has been expressed.

(c) For effects of Ca^{2+} and/or calmodulin, stimulation of adenylyl cyclase by Ca^{2+} usually requires calmodulin, except in the case of AC10 [Sacy; no calmodulin (CaM)]. All adenylyl cyclases are inhibited by high (mM) concentrations of Ca^{2+} , through competition with divalent cation required for catalysis (cf. Figs. 6 and 7). The inhibition indicated here occurs with low ($<\mu M$) concentrations of Ca^{2+} , without calmodulin, but with AC9 Ca^{2+} inhibition is with calcineurin.

(d) Ca^{2+} /calcineurin has been observed to inhibit mouse AC9 but not human AC9.

(e) AC10 (Sacy/Sac), a soluble adenylyl cyclase discovered in testes is widely distributed and functions as a HCO_3^- ion sensor. It is also stimulated by Ca^{2+} , independently of calmodulin.

(f) LTP: long term potentiation in neuronal function, PKA: cAMP-dependent protein kinase, PKC: protein kinase C, CaM: calmodulin.



Adenylyl Cyclases. Figure 4 Regulation of adenylyl cyclases by G-proteins. Abbreviations: H_s , H_i , R_s , and R_i denote hormones and receptors that lead to stimulation or inhibition, respectively, of adenylyl cyclases, C_a and C_i are active and inactive configurations of adenylyl cyclase, Fo: forskolin binding site, G_s and G_i are GTP-dependent regulatory proteins comprising their respective α_s , α_i , and $\beta\gamma$ subunits.

free cation as products (Fig. 6; transition state is depicted as $E \leftrightarrow E^*$). Cation participation in catalysis through two sites was predicted from enzyme kinetics and was later confirmed in the solved enzyme structure (Fig. 5) [3]. Available data suggest that for some isozymes substrate binding and product release are ordered and for others random. Typically, reaction velocities are considerably greater with Mn^{2+} as cation than with Mg^{2+} . Maximal velocities observed with various ATP analogs follow the order: 2'-d-5' ATP > ATP > ATP γ S > APP(NH)P > APP(CH $_2$)P. K_m values for rat brain cyclase are: $K_{MnATP} \sim 9\mu M$; K_{Mn}^{2+} , $\sim 4\mu M$; $K_{MgATP} \sim 60\mu M$; and K_{Mg}^{2+} , $\sim 860\mu M$. Notably, activation of adenylyl cyclases by hormones or by G_{α_s} , via the active enzyme configuration $GTP \cdot \alpha_s \cdot C_a$, causes a reduction in K_{Mg}^{2+} of more than an order of magnitude to $\sim 50\mu M$, without a change in K_{MgATP} .

Miscellaneous Observations

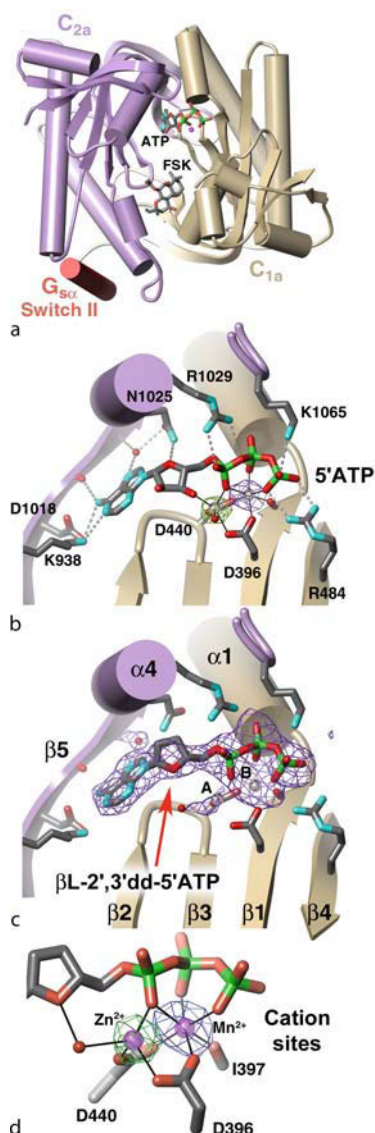
Since its first description, adenylyl cyclase has been an intensely investigated enzyme family. Consequently, numerous observations have been made of agents that affect its activity, principally in isolated membranes, but also of purified enzyme. Some of these effects would be of importance for investigators intending to work with the enzyme. First, typical enzyme preparations, whether from native or recombinant sources, are of membranes or membrane extracts that contain enzyme activities that can alter concentrations of substrate or product of adenylyl cyclases. These include activities of cyclic nucleotide phosphodiesterases, ATPases, among others, that must be taken into consideration in assays of adenylyl cyclase activities. In addition, it has been universally observed that the enzyme is protected by thiols, with β -mercaptoethanol, 2,3-dimercaptopropanol, and dithiothreitol being the most commonly used. Conversely, adenylyl cyclases are generally susceptible to oxidants, e.g. H_2O_2 , ($IC_{50} \sim 3\mu M$) and benzoquinone ($IC_{50} \sim 3\mu M$), and alkylating

agents, e.g. *N*-ethylmaleimide ($IC_{50} \sim 100\mu M$), *p*-aminophenylarsenoxide ($IC_{50} \sim 40\mu M$), *p*-aminophenyldichloroarsine ($IC_{50} \sim 80\mu M$), or *o*-iodosobenzoate ($IC_{50} \sim 10\mu M$ for AC1 against calmodulin stimulation). Not surprisingly, the crude membrane-bound enzyme is susceptible to thermal inactivation (e.g. 50% inactivation at 35° in 10 min) and purified enzyme is more labile, but protection is afforded by forskolin, substrate, $\blacktriangleright$ P-site ligands, Ca^{2+} /calmodulin (e.g. with AC1), and by $GTP\gamma S \cdot G_{\alpha_s}$. Proteases also elevate adenylyl cyclase activity. For example, acrosin, trypsin, and thrombin can cause 5–10-fold activation, and these exhibit some isozyme selectivity (AC2 > AC3 >> AC5). The basis for this activation in each case is not clear, though serine proteases are known to cleave G_{α_i} , and this could lead to indirect effects on adenylyl cyclase activity.

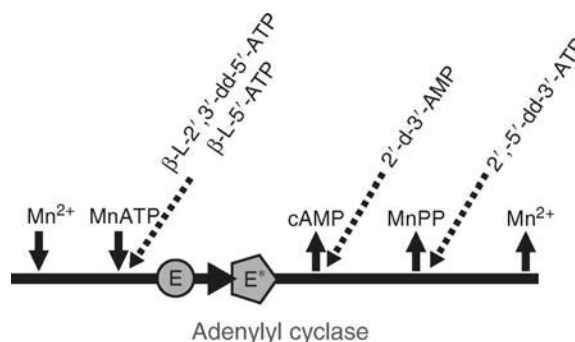
Bacterial and Other Adenylyl Cyclases

Adenylyl cyclases are found throughout the animal kingdom and serve a variety of roles. Structures of enzyme from but a few of these sources have been determined, although amino acid sequences and domain structures have been deduced for an ever increasing number. Available evidence indicates that there is little sequence homology between these adenylyl cyclases and the membrane-bound mammalian form. As is evident from the varied domain structure just of class III adenylyl cyclases (Fig. 2) [2], although the enzyme is principally membrane bound in metazoan species, it may or may not be in lower organisms. Furthermore, those forms that are membrane bound are more often than not regulated by means quite different from that described above for mammalian systems.

A comprehensive summary of these enzyme families is beyond the scope of this chapter, but a few examples are worth emphasis. The Class I adenylyl cyclases of the enterobacteria *Salmonella typhimurium*, *Yersinia pestis*, and *Escherichia coli* are membrane bound yet sequences do not give ready evidence of typical



Adenylyl Cyclases. Figure 5 Catalytic cleft and active site of a chimeric mammalian adenylyl cyclase. The cleft is formed by the pseudosymmetric interaction of enzyme cytosolic domains, C_1 and C_2 . Panel A: part of the crystal structure of the chimeric adenylyl cyclase AC5C₁•AC2C₂ with G_{α_s} , indicating binding sites for substrate (5'ATP) and forskolin (FSK). The Switch II domain of G_{α_s} interacts with the C_2 domain of adenylyl cyclase. Panel B: the catalytic active site modeled with 5'-ATP and amino acids involved in catalysis; based on structure with β -L-2',3'-dd-5'-ATP (C). Panel C: structure with β -L-2',3'-dd-5'-ATP and loci for two metal sites, A and B. Panel D: enlargement of C with Zn^{2+} (metal A) and Mn^{2+} (metal B) used in forming the crystal. Catalysis occurs with the metal catalyzed attack of the ribosyl 3'-OH group of the substrate α -phosphate. Adapted from [3].



Adenylyl Cyclases. Figure 6 Adenylyl cyclase catalytic cycle. Points during the catalytic cycle of adenylyl cyclases at which inhibition by competitive and noncompetitive nucleotides occur; E^* represents the catalytic transition state.

transmembrane domains. The enzymes comprise two principal domains, with the catalytic domain being N-terminal to a glucose-sensing regulatory domain; the enzyme is inhibited the presence of glucose. Its regulation is coordinated with that of carbohydrate permeases by the phosphoenolpyruvate:sugar phosphotransferase system. This is important for bacterial responses to changes in nutrient levels. In other bacteria, the enzyme may be regulated in response to nutrients and/or it may constitute a toxic factor in mammals, as with Class II forms of adenylyl cyclase of *Bordetella pertussis*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, or *Yersinia pestis*. These enzymes constitute the 'toxin class' of adenylyl cyclases. The well-studied adenylyl cyclases of *Bordetella pertussis* and *Bacillus anthracis* are both soluble, Ca^{2+} /calmodulin-dependent, but G-protein independent enzymes that are exported from the respective bacteria. (The adenylyl cyclase of *P. aeruginosa* is not calmodulin-dependent.) Because these enzymes are then transported into infected cells, adenylyl cyclase actually constitutes a virulence and toxic factor in mammals. The *B. pertussis* adenylyl cyclase is a large (1706 amino acids) bifunctional enzyme, the N-terminal end constituting the adenylyl cyclase activity fused to a C-terminal end exhibiting hemolytic activity and its capacity for being secreted into external medium. The *B. anthracis* adenylyl cyclase (800 amino acids), also known as 'edema factor' (EF), exhibits four domains, a signal peptide essential for protein secretion, a docking domain allowing binding to the protective antigen (PA), the adenylyl cyclase catalytic site, and a fourth region of unknown function. The *B. anthracis* adenylyl cyclase has been crystallized and its structure determined.

The Class III adenylyl cyclases are sometimes referred to as the ancestral form of the enzyme and

include numerous variants (cf. Fig. 2). Among these there are a couple of noteworthy examples. The adenylyl cyclase of *Saccharomyces cerevisiae* was the first to have been cloned and sequenced and is a prototypical Class III enzyme, with a sequence in the catalytic domain distinct from those of Class I and Class II enzymes and with the catalytic core located at the C-terminal part of the protein (Fig 2). In such yeast/fungi (e.g. *Candida albicans*) the enzyme is membrane bound and is regulated by a G-protein, in these cases Ras. As in mammalian systems it is involved in metabolic control, in mating responses, but also constitutes a virulence factor. In *C. albicans*, for example, which contains only one form of the enzyme, the cAMP signaling pathway is essential for hyphae formation and hence virulence. Sequences have been deduced for a number of enzymes of this family, including *Schizosaccharomyces pombe*, *Saccharomyces kluyveri*, *Trypanosoma brucei*, and *T. equiperdum*, *Neurospora crassa*, and *Dictyostelium discoideum*, where the adenylyl cyclase generates the cAMP that provides the signal for aggregation into a multicellular organism and the development of fruiting bodies.

Given that in many of these systems additional proteins and cofactors participate in the regulation of adenylyl cyclase activity, the full elucidation of the roles in which this enzyme activity participates in their growth, development, and function, is a long way off. This notwithstanding, the fact that the mammalian adenylyl cyclases differ so substantially from those of numerous pathogens in which the enzyme is an essential virulence factor gives motive to the idea that new classes of small molecule inhibitors of the pathogen adenylyl cyclases may be discovered that do not interact with mammalian forms of the enzyme.

Drugs

Although agents which indirectly activate or inhibit mammalian adenylyl cyclases are common and are even used in the treatment of disease, especially drugs targeting G-protein-coupled receptors, drugs acting directly on the enzyme have been less well explored. And for most compounds acting directly on adenylyl cyclases, high selectivity for specific isozymes has not been demonstrated. The main classes of such agents are derivatives of forskolin and of adenine nucleosides. Adenosine and derivatives of it have long been known to inhibit adenylyl cyclases and it became clear early on that certain modifications afforded substantially increased inhibitory potency. Notable are the approximately threefold increase in potency seen with the 2-fluorine substitution on adenine and the increases in potency seen with various modifications to the ribose moiety. The orientation of the ribose (α vs β) and the presence, orientation, or absence of hydroxyl groups clearly contribute to inhibitory potency

(Table 3). For example, arabinose and xylose differ from ribose only in the orientation of the 2'- and 3'-OH groups yet exhibit markedly different potencies. Whereas 9-(tetrahydrofuryl)-Ade (►SQ 22,536) and 9-(cyclopentyl)-Ade are without hydroxyl groups and are less potent, they offer metabolic and biochemical stability useful for many types of studies. It is, however, the removal of two of the hydroxyl groups, that elicits the largest improvement in inhibitory potency, in particular the 2',5'-dideoxy- modification (Table 3). With these improvements in potency, these cell permeable compounds, in particular ►2',5'-dd-Ado, have become useful research tools and have been used to inhibit adenylyl cyclases and to lower cAMP levels and alter function in numerous studies in isolated cells or intact tissues.

An early observation that 2'-d-3'-AMP was a more potent inhibitor of adenylyl cyclases than 2'-d-Ado suggested that the enzyme would accept substitutions at the 3'-ribose position and that phosphate was particularly well tolerated. This led to the generation of a family of 3'-phosphoryl derivatives of 2',5'-dideoxyadenosine exhibiting ever greater inhibition with the addition of an increasing number of 3'-phosphoryl groups, the most potent of which is 2',5'-dideoxyadenosine-3'-tetraphosphate (2',5'-dd-3'-A4P; Table 4) [5]. These constitute a class of inhibitors historically referred to as ►P-site ligands that caused inhibition of adenylyl

Adenylyl Cyclases. Table 3 Nucleoside inhibitors of adenylyl cyclase. Assays were with a detergent-dispersed adenylyl cyclase from rat brain and were with 100 μ M 5'ATP and 5 mM $MnCl_2$ as substrates

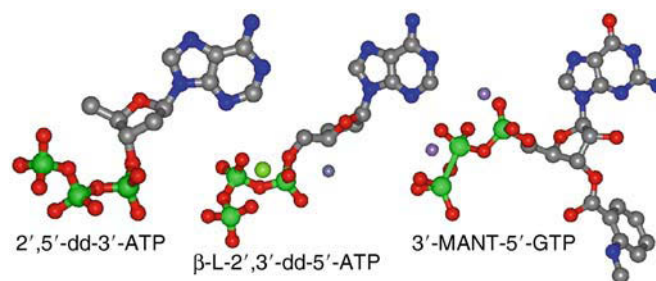
Nucleoside	IC ₅₀ (μ M)
β -Adenosine	82
α -Adenosine	>300
9-(arabinose)-Ade	30
9-(xylose)-Ade	3.2
9-(tetrahydrofuryl)-Ade	20
9-(cyclopentyl)-Ade	100
β -2'-d-Ado	15
β -3'-d-Ado (cordycepin)	13
β -2'-d-Xyl-Ade	15.5
β -2'-d-2-F-Ado	4.6
α -2'-d-2'-F-Ado	>100
β -2',3'-dd-Ado	9
β -2',5'-dd-Ado	2.8
β -2',5'-dd-Xyl-Ade	16.4
β -2',5'-dd-2-F-Ado	0.89
α -2',5'-dd-2-F-Ado	>100
β -2',5'-dd-2,5'-di-F-Ado	0.98
α -2',5'-dd-2,5'-di-F-Ado	29

Adenylyl Cyclases. Table 4 *Nucleotide inhibitors of adenylyl cyclase.* Enzyme source and assay conditions were as for Table 3. Values obtained for 3'-ATP are overestimations due to the formation of 2':3'-cAMP from 3'-ATP that occurs nonenzymatically in the presence of divalent cation

Adenine nucleoside 3'-phosphates (IC ₅₀ s μM)			
3'-phosphate	Ado	2'-d-Ado	2',5'-dd-Ado
None	82	15	2.7
3' ~P	8.9	1.2	0.46
3' ~PP	3.9	0.14	0.1
3' ~PPP	2	0.09	0.04
3' ~PPPP	–	0.011	0.0074
3' ~PS	–	3.1	0.6
Substrate analogs (IC ₅₀ s μM)			
β-L-5'-AMP		200	
β-L-2',3'-dd-5'-AMP		62	
β-D-5'-AP(CH ₂)PP		30	
β-L-5'-ATP		3.2	
β-D-2',3'-dd-5'-ATP		0.76	
β-L-2',3'-dd-5'-ATP		0.024	
Acyclic 9-substituted-Adenines (IC ₅₀ s μM)			
PMEA		65	
PMEApp		0.17	
PMEAp(NH)p		0.18	
PMPA		6.3	
PMPApp		0.5	
2'- and 3'-Substituted-5'-NTPs (IC ₅₀ s μM)			
2'(3')-MANT-5'-GTPγS		0.02	
2'(3')-MANT-5'-ITPγS		0.039	
2'(3')-MANT-5'-ATP		0.064	
3'-MANT-2'-d-5'-ATP		0.14	
3'-d-2'-MANT-5'ATΠ		0.26	
3'-7M4AMC-2'-d-5'-ATP		0.36	
3'-Dansyl-2'-d-5'-ATP		3.21	
Fluorescent-phosphoryl-derivatives (IC ₅₀ s μM)			
2',5'-dd-3'-ATP-(γ-7A4AMC)		0.166	
2',5'-dd-3'-ATP-(γ-7M4AMC)		0.88	
2',5'-dd-3'-ADP-(γ-7M4AMC)		1.65	

cyclase that was kinetically either noncompetitive or uncompetitive (cf. Fig. 6). This implied binding of the inhibitor with either a different locus or different configuration than substrate. As it developed, these are configuration selective inhibitors and they provide an exquisite means for inhibition of this signal transduction pathway. We know now that most membrane-bound forms of the mammalian adenylyl cyclase are inhibited by adenine nucleosides and their 3'-polyphosphates derivatives. Inhibition by these ligands is conserved with varying sensitivity in all isozymes, save AC10 and those of bacteria.

Probably all adenylyl cyclases are inhibited competitively by substrate analogs, which bind at the site and to the enzyme configuration with which cation-ATP binds (cf. Fig. 4). One of the best competitive inhibitors is β-L-2',3'-dideoxyadenosine-5'-triphosphate (β-L-2',3'-dd-5'-ATP; Table 4) [4], which allowed the identification of the two metal sites within the catalytic active site (cf. Fig. 4) [3]. This ligand has also been labeled with ³²P in the β-phosphate and is a useful ligand for reversible, binding displacement assays of adenylyl cyclases [4]. The two inhibitors, ►2',5'-dd-3'-ATP and β-L-2',3'-dd-5'-ATP, are comparably potent



Adenylyl Cyclases. Figure 7 Structures of potent inhibitors of adenylyl cyclase. Structures for 2',5'-dd-3'-ATP ($IC_{50} \sim 40$ nM; noncompetitive inhibitor), β -2',3'-dd-5'-ATP with Mg^{2+} and Zn^{2+} ($IC_{50} \sim 24$ nM; competitive inhibitor), and 3'-MANT-GTP with Mn^{2+} ($IC_{50} \sim 90$ nM; competitive inhibitor) are from coordinates obtained for these compounds in respective crystal structures with AC5C₁•AC2C₂. Divalent cations are indicated: Mn^{2+} : purple, Mg^{2+} : green, Zn^{2+} : blue. The 3'-MANT-group fits into a hydrophobic pocket of the enzyme. Note the difference in contortion of the phosphate chains in these structures relative to positions for divalent cation.

(Table 4), but inhibit adenylyl cyclase by conformationally distinct mechanisms (cf. Fig. 6) by binding within the catalytic cleft in unique structures (Fig. 7).

It has been known for some time that the enzyme tolerated large substitutions to the 3'-ribose position. This was taken advantage of with the development of 2' (3')-O-MANT-derivatives of nucleoside 5'-triphosphates [6] (Table 4). It was surprising, though, that potent inhibition was seen with bases other than adenine, implying that base specificity is less stringent than had been generally assumed. Subsequently, fluorescent derivatives have been made with different fluorophores at 2'- and 3'-positions. 3'-Substitutions showed advantage over corresponding 2'-substitutions and 2'(3')-O-MANT-substitutions were clearly preferable to coumarin and dansyl derivatives, but followed the order of guanosine $\geq$ inosine $>$ adenosine (Table 4). Fluorescent phosphoryl derivatives were also well tolerated, in particular the 7-amino-coumarin. These fluorescent ligands have opened possibilities for investigations of adenylyl cyclase structure, activity, and interactions with other substances not heretofore possible.

Although the 3'- and 5'-polyphosphate derivatives mentioned above exhibit exquisite inhibitory potency these compounds are not cell permeable. To take advantage of the potency of such derivatives for studies with intact cells and tissues, there are two possibilities. One is chemically to protect the phosphate groups from exonucleotidases that also allows the compound to transit the membrane intact. The other is to provide a precursor molecule that is cell permeable and is then metabolized into an inhibitor by intracellular enzymes. The general term for such a compound is ►**prodrug**; nucleotide precursors are also referred to as ►**pronucleotides**. Families of protected monophosphate derivatives were synthesized, based on β -L- and β -D-2',5'-dd-3'-AMP, β -L-2',3'-dd-5'-AMP, and the acyclic 9-substituted adenines, ►**PMEA** and ►**PMPA**. Protective substituents were: (i) -(S-pivaloyl-2-thioethyl)=

Adenylyl Cyclases. Table 5 *Prodrug inhibition of [³H] cAMP formation in intact cells*. Cells were prelabeled for 2 h with [³H]adenine before 50 μ M forskolin and pronucleotides were added. After a 15 min incubation the newly formed [³H]cAMP was extracted and quantified as in (7)

Pronucleotide	OB-1771 Preadipocytes	THP1 Monocytes
	IC_{50} (nM)	
2',5'-dd-3'-AMP-bis(Me-SATE)	6.7	260
2',5'-dd-2F-Ado-3'-P-bis(Me-SATE)	9.8	110

(*t*-Bu-SATE-); (ii) -S-acetyl-2-thioethyl)=(Me-SATE-); (iii) -(S-benzyl-2-thioethyl)=(Ph-SATE-); (iv) -cyclosalicyl=(H-Sal-); and (v) -3-methyl-cyclosalicyl=(Me-Sal-). Although triphosphate forms of each of the precursor compounds inhibit isolated adenylyl cyclases with IC_{50} s in the nanomolar range, only protected forms of 2',5'-dd-3'-AMP inhibited cAMP formation in intact cells [7]. Of these the SATE-derivatives proved the most effective. None of the pronucleotide forms of 2',5'-dd-3'-AMP inhibited adenylyl cyclase *per se*, whether isolated from rat brain or OB1771 cells. Nor were identifiable extracellular metabolites of these agents responsible for the drugs' blocking effects on intact cells. These compounds exhibit all the hallmarks of prodrugs. They are taken up, are deprotected, and are converted to extremely potent inhibitors of adenylyl cyclase, *but only by intact cells and tissues*. These prodrugs have been used to block cAMP formation in isolated cells and intact tissue and elicit functional effects (Table 5). For example, pretreatment of isolated rat atria with 1 μ M 2',5'-dd-3'-AMP-bis(*t*-Bu-SATE) completely blocked the positive chronotropic effects of 1 μ M epinephrine. It is likely that pronucleotide inhibitors of adenylyl cyclases

will find applications in many intact cell systems, as an additional upstream block of the adenylyl cyclase-cAMP-PKA signaling cascade, in biochemical, pharmacological, and potentially even therapeutic contexts.

Abbreviations Used Within the Text

Ado, adenosine
cAMP, adenosine-3':5' monophosphate
2'-d-Ado, 2'-deoxyadenosine
2'-d-2-F-Ado, 2'-deoxy-2-fluoro-adenosine
3'-d-Ado, 3'-deoxyadenosine (cordycepin)
2',5'-dd-Ado, 2',5'-dideoxyadenosine
2',3'-dd-Ado, 2',3'-dideoxyadenosine
2',5'-dd-2-F-Ado, 2',5'-dideoxy-2-fluoro-adenosine
2',5'-dd-2,5'-di-F-Ado, 2',5'-dideoxy-5'-fluoro-2-fluoro-adenosine
9-CP-Ade, 9-(cyclopentyl)-adenine
9-THF-Ade, 9-(tetrahydrofuryl)-adenine (SQ22,536)
9-Ara-Ade, 9-(arabinofuranosyl)-adenine
9-Xyl-Ade, 9-(xylofuranosyl)-adenine
2'-d-Xyl-Ade, 9-(2-deoxyxylosyl)-adenine
2',5'-dd-Xyl-Ade, 9-(2,5-dideoxyxylosyl)-adenine
2'-d-3'-AMP, 2'-deoxyadenosine-3'-monophosphate
2'-d-3'-ADP, 2'-deoxyadenosine-3'-diphosphate
2'-d-3'-ATP, 2'-deoxyadenosine-3'-triphosphate
2'-d-3'-AMPS, 3'-(thiophosphoryl)-2'-deoxyadenosine
2',5'-dd-3'-AMP, 2',5'-dideoxyadenosine-3'-monophosphate
2',5'-dd-3'-ADP, 2',5'-dideoxyadenosine-phosphate-3'-diphosphate
2',5'-dd-3'-ATP, 2',5'-dideoxyadenosine-3'-triphosphate
2',5'-dd-3'-A4P, 2',5'-dideoxyadenosine-phosphate-3'-tetraphosphate
2',5'-dd-3'-AMPS, 3'-(thiophosphoryl)-2',5'-dideoxyadenosine
5'-APP(CH₂)P, adenosine 5'-(β-(methylene)-triphosphate
β-L-5'-ATP, β-L-adenosine-5'-triphosphate
β-L-2',3'-dd-5'-ATP, β-L-2',3'-dideoxyadenosine-5'-triphosphate
PMEA, 9-[(2-phosphonylmethoxy(ethyl))-adenine
PMEApp, 9-[(2-diphosphorylphosphonylmethoxy(ethyl))-adenine
PMEAp(NH)p, 9-[(2-iminodiphosphorylphosphonylmethoxy(ethyl))-adenine
PMPA, 9-[(2-phosphonylmethoxy)propyl]-adenine
PMPApp, 9-[(2-diphosphorylphosphonylmethoxy(propyl))-adenine
2',5'-dd-3'-AMP-bis(Me-SATE), 2',5'-dideoxyadenosine-3'-(acetyl-2-thioethyl)-phosphate
2',5'-dd-3'-AMP-bis(*t*-Bu-SATE), 2',5'-dideoxyadenosine-3'-(pivaloyl-2-thioethyl)-phosphate
2',5'-dd-3'-AMP-bis(Ph-SATE), 2',5'-dideoxyadenosine-3'-(phenyl-2-thioethyl)-phosphate

2',5'-dd-2F-Ado-3'-P-bis(Me-SATE), 2',5'-dd-2-fluoro-adenosine-3'-(acetyl-2-thioethyl)-phosphate
MANT-5'-GTPγS, 3'-(2')-O-*N*-methylantraniloyl-guanosine-5'-[γ-thio]triphosphate
MANT-5'-ITPγS, 3'-(2')-O-*N*-methylantraniloyl-inosine-5'-[γ-thio]triphosphate
MANT-5'-ATP, 3'-(2')-O-*N*-methylantraniloyl-5'-ATP
MANT-5'-GTP, 3'-(2')-O-*N*-methylantraniloyl-5'-GTP
3'-MANT-2'-d-5'-ATP, 3'-O-*N*-methylantraniloyl-2'-deoxy-5'-ATP
3'-7M4AMC-2'-d-5'-ATP, 3'-(7-methoxy-4-aminomethylcoumarin)-2'-deoxy-5'-ATP
3'-(Dansyl)-2'-d-5'-ATP, 3'-(dansyl)-2'-deoxy-5'-ATP
2',5'-dd-3'-ADP-(β-7M4AMC), 2',5'-dideoxyadenosine-β-(7-methoxy-4-aminomethyl-coumarin)}-3'-diphosphate
2',5'-dd-3'-ATP-(γ-7M4AMC), 2',5'-dideoxyadenosine-γ-(7-methoxy-4-aminomethyl-coumarin)}-3'-triphosphate
2',5'-dd-3'-ATP-(γ-7A4AMC), 2',5'-dideoxyadenosine-γ-(7-amino-4-aminomethyl-coumarin)}-3'-triphosphate

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ADH

Antidiuretic Hormone.

► Vasopressin/Oxytocin

ADHD

Attention Deficit Hyperactivity Disorder.

► Psychostimulants

Adhesion Molecules

Adhesion molecules are transmembrane proteins, which through their extracellular part mediate the interaction of cells with other cells or with extracellular components like the extracellular matrix. On the basis of structural and functional similarities, most adhesion molecules can be grouped into families such as cadherins, integrins, selectins, the immunoglobulin superfamily or the syndecans. While some adhesion molecules are passive in their adhesive function, the adhesiveness of other adhesive molecules can be regulated. Some adhesive proteins are very similar to receptors, in that they not only bind other molecules with high selectivity and affinity, but are also able to transduce the binding into an intracellular signal.

- Integrins
- Cadherins
- Table appendix: Adhesion Molecules
- Anti-integrins

Adipocyte

Adipocyte is the term for a fat cell which is used to store energy in the form of triacylglycerols. There are two different types of adipocytes. (i) White adipocytes contain a large lipid droplet and secrete different adipocytokines like adiponectin, and resistin. The lipid may account to 90% of cell mass. (ii) Brown adipocytes contain lipid droplets scattered throughout the single cell and are used to generate heat in a process known as nonshivering thermogenesis.

Fibroblasts, which are undifferentiated pre-adipocytes, can be stimulated and converted into adipocytes.

- Fatty Acid Transport Proteins
- Adipokines

Adipocytokines

► Adipokines

Adipokines

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Synonyms

Adipocytokines

Definition

The term adipokine refers to any protein secreted from adipocytes [1]. Collectively, the various adipokines form the ‘adipokinome’ which together with the lipid moieties secreted from fat cells (e.g. fatty acids, cholesterol, retinol) constitute what can be referred to as the ‘secretome’ of adipocytes. Most adipokines are also secreted from other cell types in other organs, but one in particular – adiponectin – is considered to be exclusive to adipocytes.

Basic Characteristics

► **White adipose tissue**, or white fat, has been traditionally viewed essentially as an organ of fuel storage. Around half of the total cell content of adipose tissue is made up of mature ► **adipocytes**, the other cell types including fibroblasts, preadipocytes, macrophages and endothelial cells. Lipids are stored in mature white adipocytes as triacylglycerols in the form of a single large droplet. This gives a unilocular appearance under the microscope, and lipid can constitute up to 90% of the cell mass. Triacylglycerols are deposited in the tissue at a time of nutritional excess to be released during periods of negative energy balance – such as fasting and long-term starvation. Lipids allow fuel to be stored at a high energy density since their calorific value is twice that of carbohydrates, and because in contrast to carbohydrate they can be stored with little associated water.

Additional roles traditionally recognised for white adipose tissue include thermal insulation (as in the blubber of sea mammals) and mechanical protection to internal organs. However, in recent years it has become apparent that the function of white adipose tissue is much more extensive. This follows from the discovery that it is a major endocrine organ, secreting a diverse

range of protein hormones and other protein factors – the ►adipokines [1–3]. These secreted protein signals and factors were initially called adipocytokines, but the term adipokines is now generally used. This reflects the fact that the name ‘adipocytokine’ implies that the proteins are cytokines, or cytokine-like. While this is true of some, it is not so for the majority.

The first protein factor to be identified as being secreted from adipocytes was the enzyme lipoprotein lipase, which is responsible for the breakdown of circulating triacylglycerols (largely in the form of lipoproteins) to release free fatty acids and glycerol; however, no special significance was attributed to this particular secretion. The fatty acids are then taken up into the adipocytes and re-esterified to triacylglycerol. The next adipocyte secreted protein to be identified was adipsin. This is a complement-related factor which was initially thought to play an important role in the control of energy balance and body weight as a lipostatic signal, although this was quickly recognised not to be the case. In the early 1990s adipocytes were then found to express and secrete tumour necrosis factor- α (►TNF α), a major pro-inflammatory cytokine. TNF α was immediately linked to the development of insulin resistance in fat cells, but its actions are now considered to be rather more extensive. It plays, for example, a pivotal role in the regulation of the production of a number of other adipokines, particularly those related to ►inflammation.

Leptin and Adiponectin

The protein factor whose identification induced the radical change in our understanding of the physiological role of adipose tissue was the hormone ►leptin (Greek: *leptos* – thin or small). This was discovered in 1994 as the product of the *OB (LEP)* gene, mutations in which result in the profound ►obesity of the obese (*ob/ob*) mouse [1–4]. Mutations in the leptin gene have subsequently been identified in humans, and as in mice they are associated with extreme obesity. Leptin is a key satiety factor, providing a signal from adipocytes to the hypothalamus in the neuroendocrine regulation of appetite [3, 4]. In practise, leptin is now recognised to be a pervasive hormone with multiple functions, including as a signal in the maturation of the reproductive system, in immunity and in insulin secretion and glucose utilisation. Although leptin is produced in a number of different cells and organs, the major site of production is the adipocyte, with the amount of body fat being the primary determinant of the circulating level of the hormone. There is, however, acute regulation of leptin production with an important role for insulin and the sympathetic nervous system. Indeed, the sympathetic system provides a negative feedback loop from the brain to adipocytes in the control of leptin production.

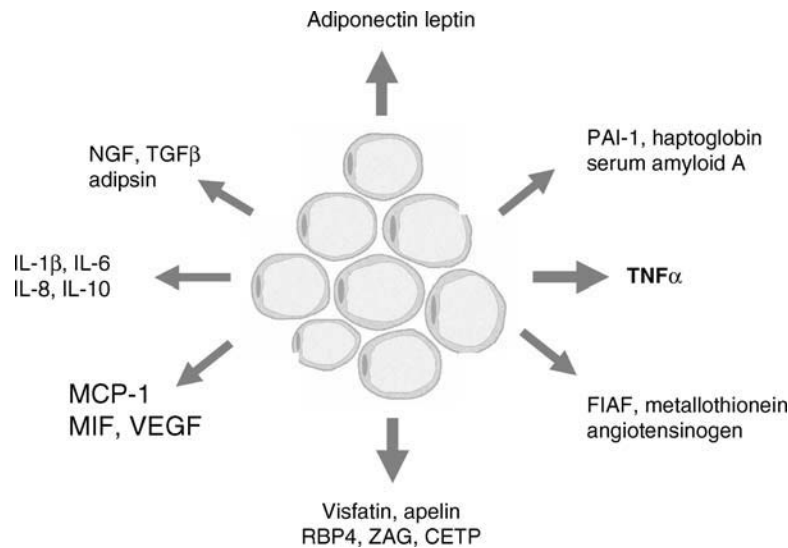
The discovery of leptin resulted in white adipose tissue being recognised as a major endocrine organ. Indeed, in many cases – particularly the obese – it is the largest endocrine organ in the body, amounting to up to 50% or more of total tissue mass. Following the identification of leptin, a rapidly expanding list of other protein hormones and factors secreted from adipocytes has been identified (Fig. 1). The total number of these adipokines is now in excess of 50 distinct molecular entities [1, 2].

The second major hormone, identified to be secreted from adipocytes, was ►adiponectin [3]. This factor was discovered by several groups in the mid 1990s and is also known as Acrp30, AdipoQ, ApM1 and GBP28. Adiponectin (the most widely used name) appears to be secreted exclusively by adipocytes, in contrast to the other adipokines. Adiponectin is involved in a wide range of functions. These include important roles in modulating insulin sensitivity, in inflammation (anti-inflammatory) and atherogenesis. Adiponectin circulates at high concentrations in the blood, occurring in high molecular weight polymeric forms. In marked contrast to other adipokines, its production and circulating levels fall in obesity.

Inflammation

A number of adipokines are linked to inflammation and immunity (Fig. 1). This includes both leptin and adiponectin, and also a number of other key inflammatory proteins, particularly cytokines and chemokines [1]. The cytokines and chemokines encompass interleukin-1 β (IL-1 β), IL-6, IL-10, TNF α , monocyte chemoattractant protein-1 (MCP-1), and macrophage migration inhibitory factor (MIF). Other major inflammation-related adipokines include nerve growth factor (NGF), and acute phase proteins such as serum amyloid A and haptoglobin. In addition, adipocytes secrete plasminogen activator inhibitor-1 (PAI-1), which is an important thrombotic factor as well as an acute phase protein.

The wide range of inflammation-related factors that adipocytes secrete is linked to the inflammatory response that the tissue exhibits in obesity [1]. Obesity in general, like an increasing number of other diseases, is characterised by a state of mild chronic inflammation, and adipose tissue plays a central role in this. The production of most inflammation-related adipokines increases markedly in obesity and there is an elevated circulating level of a number of these factors as well as of other inflammatory markers such as C-reactive protein (CRP). The increased production of inflammatory adipokines (and decreased production of adiponectin with its anti-inflammatory action) in the obese is considered to play a critical role in the development of the obesity-associated pathologies, particularly type 2 diabetes and the ►metabolic syndrome [1].



Adipokines. Figure 1 Major proteins – adipokines – secreted from white adipocytes. CETP, cholesteryl ester transfer protein; FIAF, fasting-induced adipose factor (angiopoietin like protein-4) IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MIF, macrophage migration inhibitory factor; NGF, nerve growth factor; PAI-1, plasminogen activator inhibitor-1; RBP4, retinol binding protein-4; TGF β , transforming growth factor- β ; TNF α , tumour necrosis factor- α ; VEGF, vascular endothelial growth factor; ZAG, zinc α 2-glycoprotein.

An important component of the inflammatory state in adipose tissue in obesity comes from the infiltration of the tissue by macrophages. These are likely to be attracted through the secretion by adipocytes of MCP-1 and MIF. The macrophages in turn secrete factors which both directly add to the total production of inflammatory agents by adipose tissue and also catalyse the production of such agents from adipocytes – and perhaps preadipocytes as well.

The wide range of inflammatory and immune factors secreted by adipocytes has led to the view that there are many similarities between these cells and cells of the immune system.

Other Adipokines and Metabolic Processes

In addition to the regulation of energy balance and the mounting of an inflammatory response, adipokines are involved in a number of other physiological and metabolic processes [3, 4]. These include lipid metabolism (e.g. retinol binding protein, cholesteryl ester transfer protein), the control of blood pressure through the renin-angiotensin system (e.g. angiotensinogen), vascular haemostasis (e.g. PAI-1), angiogenesis, glucose homeostasis and stress responses. Several factors are involved in angiogenesis and these not only include the key angiogenic factor vascular endothelial growth factor (VEGF), but also leptin and angiopoietin-like protein 4/fasting-induced adipose factor (Angptl4/FIAF). Leptin and adiponectin – and perhaps IL-6 and MCP-1 – play an important role in glucose homeostasis and insulin sensitivity.

The current view is that through the various adipokines, adipocytes and adipose tissue are implicated in a wide range of physiological functions in a manner that far transcends the original simple paradigm of fuel storage. Indeed, there is extensive cross-talk between adipocytes and other cells and organs, including the brain, skeletal muscle and bone. Within adipose tissue itself, there is cross-talk between adipocytes, macrophages and preadipocytes, particularly in relation to the inflammatory response. The discovery of the adipokines and the endocrine role of adipose tissue has led to parallel developments with skeletal muscle; myocytes are now recognised to secrete protein factors also, in particular IL-6, leading to the concept of ‘myokines’.

Drugs

Little attempt has been made to develop drugs targeted specifically to white adipose tissue and the production of adipokines. It is likely, however, that there will be an increasing emphasis on this approach to the pharmacological treatment of obesity-related diseases, given the current views on the centrality of the adipokines to these disorders. It is, of course, the diseases that obesity leads to, rather than obesity itself, that constitute the main medical challenge.

There has been considerable focus on the development of drugs that lead to a reduction in the total amount of adipose tissue. These include agents targeted at limiting fat absorption, the inhibition of appetite, and the stimulation of energy expenditure (thermogenesis) – or a combination thereof. The best example of drugs,

targeted specifically to adipocytes, are the β_3 -adrenoceptor agonists such as the first generation compound BRL-37344 [(RR + SS)-(+)-4-[2-(2-(3-chlorophenyl)-2-hydroxyethyl)amino)propyl] phenoxyacetate]. These stimulate lipolysis and thermogenesis (in brown adipose tissue), but their use in humans has been problematic because the β_3 -adrenoceptor appears less important in man than it is in rodents and because of the differences between the human and the rodent (on which early studies were directed) receptors. Such drugs in practice also directly modulate the production of certain adipokines; a potent example is leptin, the expression and production of which is strongly inhibited by β_3 -adrenoceptor agonists.

Currently, three drugs are available clinically to treat obesity – Orlistat, Reductil (sibutramine) and Rimonabant. Orlistat inhibits pancreatic lipase, thereby reducing the digestion and subsequent absorption of fatty acids. Sibutramine, which is a serotonin and noradrenergic reuptake inhibitor, suppresses appetite and stimulates energy expenditure. Rimonabant, the most recently introduced anti-obesity drug, is targeted at the endocannabinoid system, acting as an antagonist of the CB1 receptor. Rimonabant inhibits appetite and probably also stimulates expenditure. [5] It is increasingly clear that this drug also interacts with adipose tissue, either indirectly or directly, CB1 receptors being present in the tissue [5]. Adiponectin production appears to be stimulated by Rimonabant and this has raised the possibility that this drug and other CB1 antagonists and agonists might have specific effects on adipokine synthesis, particularly those linked to inflammation and insulin sensitivity. As such, CB1 antagonists may have a potential role in the treatment of the metabolic syndrome.

The established example of current drugs which directly affect adipokine production is the ►thiazolidinediones (such as pioglitazone and rosiglitazone) which were developed as anti-diabetic agents. These compounds, which interact with the ►PPAR γ nuclear receptor, have major effects on the production of several adipokines. Thus production of leptin is strongly downregulated, whereas that of adiponectin is upregulated. Importantly, PPAR γ activators have an extensive anti-inflammatory action. As a consequence, the production of several inflammation-related adipokines has been shown to be inhibited by treatment with TZDs.

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Adiponectin

A major adipokine, molecular weight 28,000 Da (monomeric form), that is secreted only from adipocytes. It exists at high levels in the plasma and has a number of functions, including an important role in insulin sensitivity, inflammation (anti-anti-inflammatory action) and atherogenesis. Unlike most adipokines, the plasma levels fall in obesity.

- Adipokines
- Arp-activated Protein Kinase

Adipose Tissue

The adipose organ consists of two distinct tissues – brown and white adipose tissue. Brown adipose tissue is specialised for thermoregulatory heat generation (thermogenesis) through the presence of the tissue-specific mitochondrial uncoupling protein-1 (UCP-1); only limited amounts are present in most humans. White adipose tissue is the major fuel storage organ in mammals and birds, the key cell type (~50% of total cell content) being the adipocytes which store large amounts of triacylglycerol; the other cell types are fibroblasts, preadipocytes, macrophages and endothelial cells.

- Adipokines

Adrenal Gland

The adrenal gland is a flattened gland situated above each kidney, consisting of a cortex (outer wall) that

secretes important steroid hormones and a medulla (inner part) that secretes adrenaline (epinephrine) and noradrenaline (norepinephrine).

► Glucocorticoids

Adrenaline

Adrenaline (epinephrine) is a catecholamine, which is released as a neurotransmitter from neurons in the central nervous system and as a hormone from chromaffin cells of the adrenal gland. Adrenaline is required for increased metabolic and cardiovascular demand during stress. Its cellular actions are mediated via plasma membrane bound G-protein-coupled receptors.

► α -Adrenergic System

► β -Adrenergic System

Adrenergic Receptor

Also called adrenoreceptors, are a class of G protein-coupled receptors with a widespread expression in a broad spectrum of different organs and tissues. Adrenergic receptors are activated by their endogenous agonist ligands epinephrine and norepinephrine which belong to the catecholamine transmitters. Adrenergic receptors regulate a range of physiological parameters such as blood pressure and heart rate in a very rapid manner; these rapid changes in parameters such as heart rate are essential elements of physiological defense reactions (“fight and flight”).

► α -Adrenergic System

► β -Adrenergic System

► Trace Amines

α -Adrenergic Receptors

► α -Adrenergic System

α -Adrenergic System

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Synonyms

α -Adrenergic receptors; α -Adrenoceptors

Definition

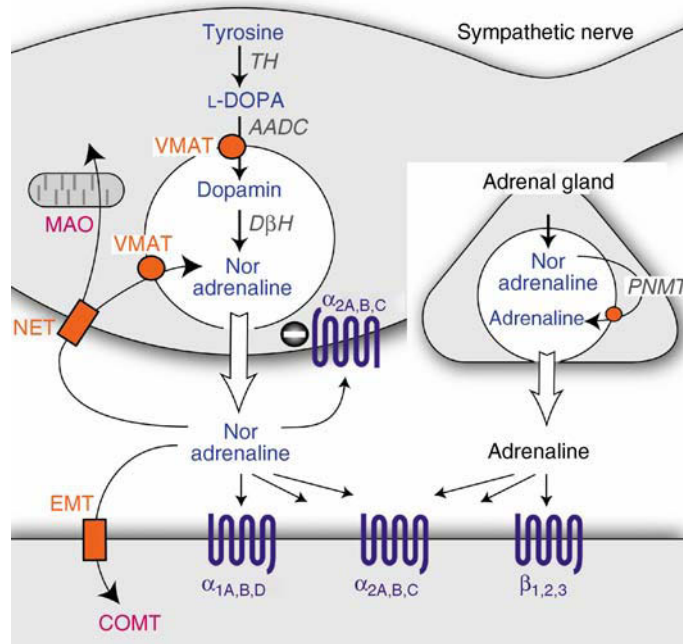
The α -adrenergic system consists of six subtypes of membrane receptors which mediate part of the biological actions of the catecholamines adrenaline and noradrenaline. α -Adrenergic receptors are important regulators of smooth muscle cell contraction (α_1 -adrenergic receptors) [1] and presynaptic neurotransmitter release (α_2 -adrenergic receptors) [2, 3]. In addition, adrenaline and noradrenaline activate β -adrenergic receptors, which stimulate cardiac contractility and rhythm and inhibit bronchial, vascular, and uterine smooth muscle contraction (► β -adrenergic system).

Basic Characteristics

The adrenergic system is an essential regulator that increases cardiovascular and metabolic capacity during situations of stress, exercise, and disease. Nerve cells in the central and peripheral nervous system synthesize and secrete the neurotransmitters noradrenaline and adrenaline. In the peripheral nervous system, ►noradrenaline and ►adrenaline are released from two different sites: noradrenaline is the principal neurotransmitter of sympathetic neurons that innervate many organs and tissues. In contrast, adrenaline, and to a lesser degree noradrenaline, is produced and secreted from the adrenal gland into the circulation (Fig. 1). Thus, the actions of noradrenaline are mostly restricted to the sites of release from sympathetic nerves, whereas adrenaline acts as a hormone to stimulate many different cells via the blood stream.

Together with dopamine, adrenaline and noradrenaline belong to the endogenous catecholamines that are synthesized from the precursor amino acid tyrosine (Fig. 1). In the first biosynthetic step, tyrosine hydroxylase generates ►L-DOPA which is further converted to dopamine by the aromatic L-amino acid decarboxylase (►Dopa decarboxylase). Dopamine is transported from the cytosol into synaptic vesicles by a vesicular monoamine transporter. In sympathetic nerves, vesicular dopamine β -hydroxylase generates the neurotransmitter noradrenaline. In chromaffin cells of the adrenal medulla, approximately 80% of the noradrenaline is further converted into adrenaline by the enzyme phenylethanolamine-*N*-methyltransferase.

Several mechanisms serve to terminate the biological actions of noradrenaline and adrenaline. From the



α -Adrenergic System. Figure 1 Synthesis and release of noradrenaline and adrenaline from sympathetic nerve endings (left) and from the adrenal gland (right). Noradrenaline and adrenaline are synthesized from the precursor amino acid tyrosine and are stored at high concentrations in synaptic vesicles. Upon activation of sympathetic nerves or adrenal chromaffin cells, noradrenaline and adrenaline are secreted and can activate adrenergic receptors on surrounding cells (sympathetic nerve), or they enter the blood circulation (adrenaline released from the adrenal gland). Release of noradrenaline from nerve terminals is controlled by presynaptic inhibitory α_2 - and activating β_2 -adrenergic receptors. Actions of noradrenaline are terminated by uptake into nerve terminals and synaptic vesicles by active transporters (NET, EMT, VMAT) and by uptake into neighboring cells (not shown). Abbreviations: AADC, aromatic L-amino acid decarboxylase; COMT, catechol O-methyltransferase; DBH, dopamine β -hydroxylase; EMT, extraneuronal noradrenaline transporter; MAO, monoamine oxidase; NET, noradrenaline transporter; PNMT, phenylethanolamine-N-methyltransferase; TH, tyrosine hydroxylase; VMAT, vesicular monoamine transporter.

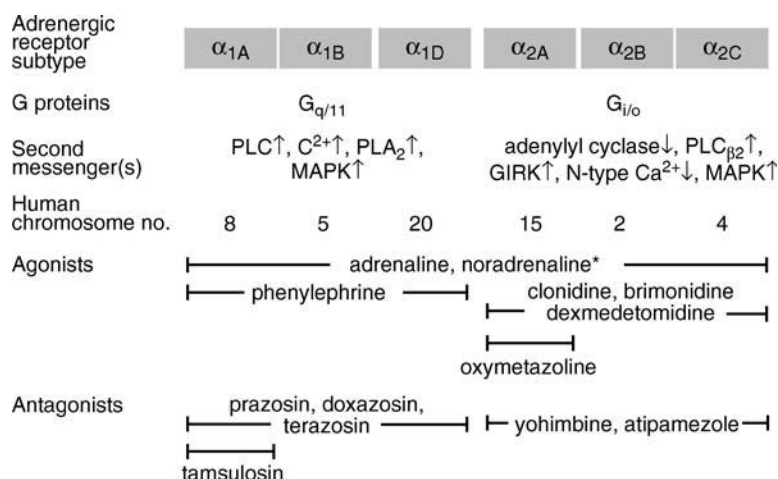
synaptic cleft, most of the released noradrenaline is recycled by reuptake into the nerve terminals via a specific **noradrenaline transporter**. This transporter is selectively blocked by cocaine, tricyclic antidepressants or selective noradrenaline reuptake inhibitors (SNRIs). After reuptake into the nerve, most of the noradrenaline is transferred into synaptic vesicles. A smaller fraction is destined for degradation by the enzymes **monoamine oxidase** (MAO, in sympathetic nerves) or **catechol-O-methyltransferase** (COMT, in neighbouring cells). COMT plays a major role in the metabolism of circulating catecholamines. MAO and COMT are widely distributed, and inhibitors of these enzymes are used for the treatment of mental depression (MAO-A inhibitor, moclobemide) or Parkinson's disease (MAO-B inhibitor, selegiline).

The biological actions of adrenaline and noradrenaline are mediated via nine different **G-protein-coupled receptors**, which are located in the plasma membrane of neuronal and nonneuronal target cells. These receptors are divided into two different groups, α -adrenergic receptors and β -adrenergic receptors (see β -adrenergic system).

The distinction between α - and β -adrenergic receptors was first proposed by Ahlquist in 1948 based on experiments with various catecholamine derivatives to produce excitatory (α) or inhibitory (β) responses in isolated smooth muscle systems. Initially, a further subdivision into presynaptic α_2 - and postsynaptic α_1 -receptors was proposed. However, this anatomical classification of α -adrenergic receptor subtypes was later abandoned.

At present, six α -adrenergic receptors have been identified by molecular cloning: three α_1 -adrenergic receptors (α_{1A} , α_{1B} , α_{1D}) and three α_2 -subtypes (α_{2A} , α_{2B} , α_{2C}) (Fig. 2). Due to the lack of sufficiently subtype-selective ligands, the unique physiological properties of these α -receptor subtypes, for the most part, have not been fully elucidated. However, recent studies in mice that carry deletions in the genes encoding for individual α -receptor subtypes have greatly advanced the knowledge about the specific functions of these receptors.

α_1 -Adrenergic receptors mediate contraction and hypertrophic growth of vascular smooth muscle cells and cardiac myocytes. The three α_1 -receptor subtypes share 75% identity in their transmembrane domains,



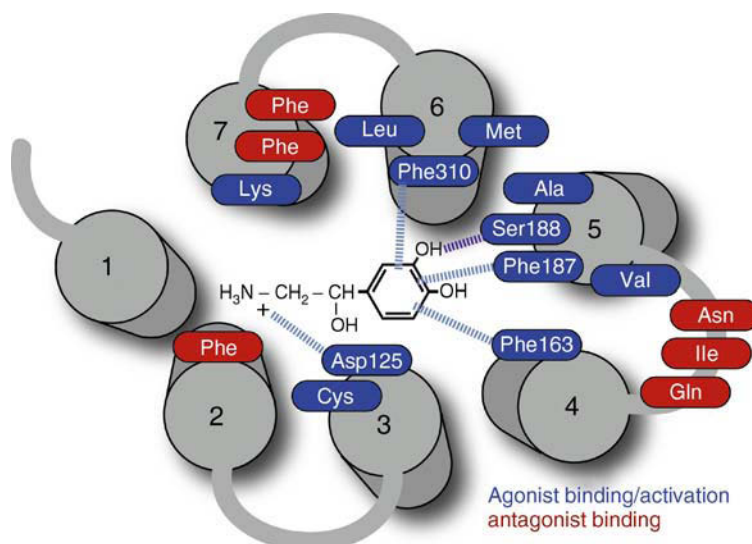
α -Adrenergic System. Figure 2 Subtypes of α -adrenergic receptors, their signaling pathways and agonist and antagonist binding profiles. The proposed topology of α_1 - and α_2 -adrenergic receptors with 7 transmembrane domains is illustrated. *Adrenaline and noradrenaline can also activate β -adrenergic receptors (see β -adrenergic system). PLA $_2$, PLC: phospholipases A, C; GIRK: G-protein-activated inwardly rectifying potassium channel, MAPK: mitogen-activated protein kinase.

whereas the degree of homology between α_1 - and α_2 -receptors is significantly smaller (35–40%). Due to discrepancies between the pharmacological subtype classification, mRNA and protein expression data and experiments with cloned α_1 -receptor subtypes, some confusion exists in the literature with respect to the assignment of α_1 -receptor subtype nomenclature. In the present terminology, α_{1A} (cloned α_{1c}), α_{1B} (cloned α_{1b}) and α_{1D} -receptors (cloned α_{1d}) can be distinguished. All three subtypes seem to be involved in the regulation of vascular tone, with the α_{1A} -receptor maintaining basal vascular tone and the α_{1B} -receptor mediating the constrictory effects of exogenous α_1 -agonists. Cardiac α_1 -receptors increase contractile force and mediate antiapoptotic and hypertrophic effects. All α_1 -receptor subtypes can activate G_q -proteins, resulting in intracellular stimulation of phospholipases C, A $_2$, and D, mobilization of Ca^{2+} from intracellular stores and activation of mitogen-activated protein kinase and PI3 kinase pathways. Mutagenesis of receptor subtypes has led to the identification of a number of amino acids involved in agonist binding and receptor activation as well as binding sites for antagonists within the receptor's binding crevice [4, 5] (Fig. 3).

Three genes encoding for α_2 -adrenergic receptor subtypes have been identified from several species, termed α_{2A} , α_{2B} , and α_{2C} , respectively (Fig. 2). The pharmacological profile of the α_{2A} -subtype differs significantly between species, thus giving rise to the pharmacological subtypes α_{2A} in humans, rabbits, and pigs and α_{2D} in rats, mice, and guinea pigs. Part of the pharmacological difference between α_{2A} - and α_{2D} -receptors can be explained by a Ser-Ala mutation in the

fifth transmembrane helix of the α_{2A} -receptor rendering this receptor less sensitive to the antagonists, rauwolscine and yohimbine. α_2 -Adrenergic receptors regulate a wide range of signalling pathways via interaction with multiple heterotrimeric $G_{i/o}$ proteins including inhibition of adenylyl cyclase, stimulation of phospholipase D, stimulation of mitogen-activated protein kinases, stimulation of K^+ currents and inhibition of Ca^{2+} currents. The three α_2 -receptor subtypes have unique patterns of tissue distribution in the central nervous system and in peripheral tissues. The α_{2A} -receptor is expressed widely throughout the central nervous system including the locus coeruleus, brain stem nuclei, cerebral cortex, septum, hypothalamus, and hippocampus. In the periphery, α_{2A} -receptors are expressed in kidney, spleen, thymus, lung, and salivary gland. The α_{2B} -receptor primarily shows peripheral expression (kidney, liver, lung, and heart) and only low level expression in thalamic nuclei of the central nervous system. The α_{2C} -receptor appears to be expressed primarily in the central nervous system (striatum, olfactory tubercle, hippocampus, and cerebral cortex), although very low levels of its mRNA are present in the kidney.

α_{2A} -, α_{2B} -, and α_{2C} -receptors are located presynaptically in order to inhibit noradrenaline release from sympathetic nerves. Activation of these receptors leads to decreased sympathetic tone, decreased blood pressure and heart rate. Central α_{2A} -receptors mediate sedation and analgesia. α_{2B} -Receptors mediate contraction of vascular smooth muscle, and in the spinal cord they are essential components of the analgesic effect of nitrous oxide. Upon stimulation by agonists, α_1 - and α_2 -receptor signalling pathways are attenuated by



α -Adrenergic System. Figure 3 Model of agonist and antagonist binding to α_1 -adrenergic receptors. The binding pocket of α_1 -receptors is depicted from an extracellular viewpoint, transmembrane domains 1–7 are numbered. Amino acids involved in agonist binding and receptor activation are depicted in blue, residues which mediate antagonist binding are shown in red (modified from 5).

several mechanisms at the receptor and postreceptor levels (see β -adrenergic system).

Drugs

Therapeutically, α_1 -receptor-mediated vasoconstriction contributes to the beneficial actions of adrenaline applied as an emergency medicine during hypotensive or anaphylactic shock. Addition of adrenaline or noradrenaline to local anaesthetics prevents diffusion of the local anaesthetic from the site of injection and thereby prolongs its action. α_1 -Receptor antagonists including prazosin, doxazosin, terazosin, and bunazosin are used to treat patients with hypertension. However, α_1 -receptor antagonists are no longer first-line antihypertensive agents since the ALLHAT clinical trial revealed that hypertensive patients taking doxazosin had a higher risk of developing congestive heart failure than patients with diuretic treatment. Tamsulosin is the first α_{1A} -receptor antagonist with selectivity for the α_{1A} -receptor over α_{1B} - and α_{1D} -subtypes. The α_{1A} -selectivity is thought to contribute to the beneficial actions of tamsulosin and alfuzosin in the treatment of benign prostate hypertrophy without lowering blood pressure.

At present, no drugs exist that can selectively activate α_2 -receptor subtypes. Clonidine stimulates all three α_2 -subtypes with similar potency. Clonidine lowers blood pressure in patients with hypertension and it decreases sympathetic overactivity during opioid withdrawal. In intensive and postoperative care, clonidine is a potent sedative and analgesic and can prevent postoperative shivering. Clonidine and its derivative brimonidine lower

intraocular pressure of glaucoma patients when applied locally. Moxonidine may have less sedative side effects than clonidine when used as an antihypertensive. It has been suggested that moxonidine activates “imidazoline receptors” instead of α_2 -receptors. The α_2 -receptor agonists oxymetazoline and xylometazoline are being used as nasal decongestants. At present, α_2 -receptor antagonists are not used in human medicine. However, in veterinary practice the α_2 -receptor antagonist atipamezole can rapidly reverse anaesthesia mediated by the α_2 -agonist medetomidine. In the future, subtype-selective drugs may greatly improve the therapy of diseases involving α_1 - or α_2 -adrenergic receptor systems.

► β -Adrenergic System

► Catechol-O-Methyltransferase and its Inhibitors

► Neurotransmitter Transporters

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β -Adrenergic System

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Synonyms

β -adrenoceptor system

Definition

The β -adrenergic system defines the effects of the ►**sympathetic system** mediated via β -adrenergic receptors (synonym: β -adrenoceptors). These are G-protein-coupled receptors primarily causing an activation of adenylyl cyclases. They mediate a plethora of cardiovascular, smooth muscle and metabolic effects. β -Adrenergic receptor antagonists are used to treat various cardiovascular diseases, including hypertension, coronary artery disease, myocardial infarction and heart failure, but also glaucoma and hyperthyroidism. β -Adrenergic receptor agonists are used primarily to treat bronchial asthma and premature labour.

Basic Characteristics

The sympathetic nervous system secretes the ►**catecholamines** noradrenaline (norepinephrine) from nerve endings and adrenaline (epinephrine) from the adrenal medulla. Noradrenaline is stored in the varicosities ($\approx$ nerve endings) of the sympathetic nervous system, often together with ATP or neuropeptide Y. Adrenaline is stored in vesicles of the chromaffin cells of the adrenal medulla together with its precursor noradrenaline at a ratio of about 4:1. Release of noradrenaline and adrenaline is subject to inhibitory control by presynaptic α_{2A} - and α_{2C} -adrenergic receptors, and to – less pronounced – stimulation via presynaptic β_2 -adrenergic receptors (see chapter on α -adrenergic system).

Adrenaline and noradrenaline act on a total of nine adrenergic receptor subtypes, three each of the α_1 -, α_2 - and β -adrenergic subfamily. The three β -adrenergic receptors are termed β_1 , β_2 and β_3 . All adrenergic receptors couple to ►**G-proteins**. G_s is the primary G-protein for all three β -adrenergic receptors and mediates activation of ►**adenylyl cyclases**, i.e. results in an increase of intracellular cAMP-levels. Activation of ►**protein kinase A** (PKA) is the main effector pathway of elevated cAMP, but cAMP can also activate cyclic-nucleotide-dependent ion channels, inhibit the metabolism of cGMP by phosphodiesterases and activate the epac proteins (exchange proteins activated by cAMP). Additional signalling pathways have been observed mainly for the β_2 -subtype, they include activation of G_i as well as stimulation of ►**MAP-kinase**

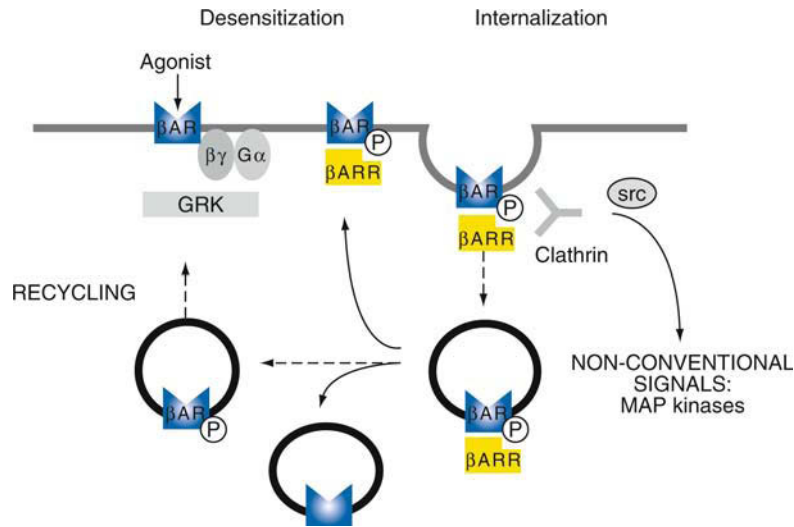
pathways (MAP-kinase cascades); activation of these “non-conventional” pathways appears to require the binding of β -arrestins to the receptors (see below). These G-protein-independent signalling pathways have been suggested by a variety of experiments, and it appears that they play a role in long-term signalling regulating cell growth [1–4].

All adrenergic receptors are heptahelical, i.e. they have seven transmembrane helices that form a ligand-binding pocket located in the central transmembrane core of the receptors. Amino acids essential for ligand binding have been mapped extensively for the β_2 -adrenergic receptor; they are located in transmembrane helices 3, 5 and 6. Receptor activation involves an agonist-induced intramolecular conformational change that causes a relative movement of the transmembrane helices 3 and 6. This is thought to lead to a rearrangement of the intracellular parts of the receptor, which couple to G-proteins, most notably the part of the third intracellular loop that is adjacent to transmembrane helix 6. These conformational changes can be directly visualized in intact cells with fluorescence techniques and occur over a few hundred milliseconds after agonist binding [5].

Recent studies indicate that – like many other receptors – G-protein-coupled receptors may form dimers, either homodimers or dimers with another type of receptor. The role of dimer formation in the cell surface expression of receptors and in their signalling and the resultant pharmacology are currently under intensive investigation [1].

In addition to agonist-induced activity, many receptors, including the β -adrenergic receptors, display spontaneous or constitutive activity. This means that the unoccupied receptor has some likelihood to adopt an active conformation, couple to G-proteins and generate an intracellular signal. Some compounds classified as antagonists (e.g. propranolol) can suppress constitutive activity and are therefore termed inverse agonists. Constitutive activity is more pronounced for the β_2 -than the β_1 -subtype, but has not yet been investigated for the β_3 -receptor.

The agonist-induced conformational change not only causes receptor activation and generation of an intracellular signal, but also a number of biochemical processes that dampen the signal and cause desensitization of the receptor [2] (Fig. 1). These include (i) phosphorylation of the receptor by members of the G-protein-coupled receptor kinase (GRK) family, followed by binding of β -arrestins, which prevents further activation of G-proteins, (ii) phosphorylation by protein kinases A and C (PKA and PKC), which directly impairs G-protein-coupling, (iii) translocation of the receptors into clathrin-coated pits and internalization into endosomes. Binding of β -arrestins and perhaps also movement of



β -Adrenergic System. Figure 1 Desensitization, internalization and recycling of β -adrenergic receptors. Activation of β -adrenergic receptors causes their phosphorylation by members of the GRKs. Cytosolic β -arrestins then bind to the phosphorylated receptors and prevent further interaction with G-proteins. β -Arrestin-bound receptors assemble in clathrin-coated pits, where the complex appears to interact with other proteins, including dynamin and src-kinase. This leads (i) to the activation of non-conventional signalling pathways (raf-kinases, MAP-kinases, JNK-kinases), and (ii) to the internalization of the receptors to endosomes. Endosomal receptors become either dephosphorylated and recycle back to the cell surface; some endosomal receptors undergo lysosomal degradation.

receptors into clathrin-coated pits appear also to be required for a subsequent “wave” of non-conventional signalling, such as activation of MAP-kinases [3]. Most internalized receptors are recycled back to the cell surface, but some are degraded. The regulatory processes described above are most pronounced for the β_2 -subtype, and least for the β_3 -subtype; they have also been demonstrated for other G-protein-coupled receptors, e.g. the α_2 -adrenergic receptors. In addition to these regulatory processes at the level of the receptor protein, the receptor mRNA-levels can be down-regulated, at least in part by destabilization via mRNA-binding proteins; this results in reduced receptor synthesis. Whereas the phosphorylation processes and the subsequent receptor desensitization can occur over a few minutes of agonist exposure, receptor down-regulation takes many hours and may even take days to reach a new steady-state level [2].

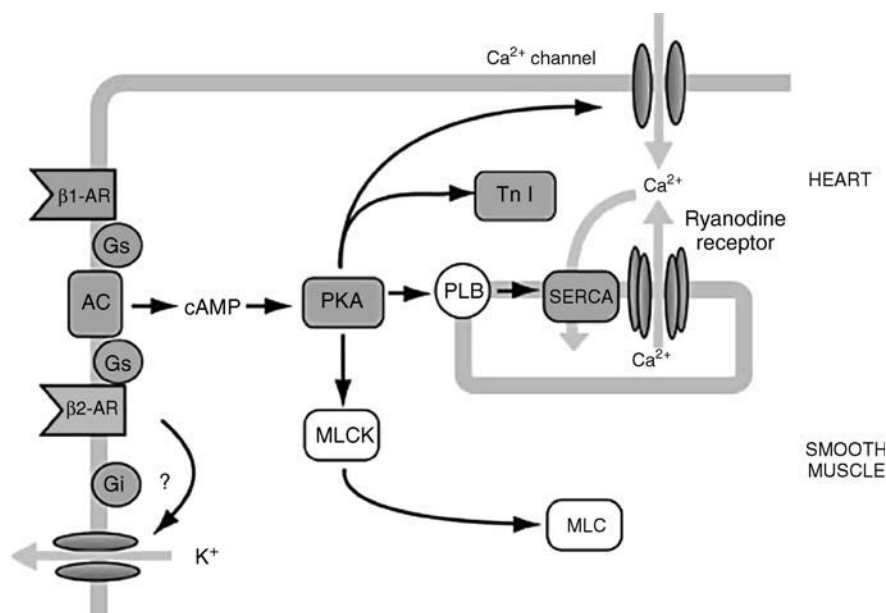
β -Adrenergic receptors mediate a plethora of cardiovascular, smooth muscle and metabolic effects (Fig. 2). Cardiac β_1 -adrenergic receptors increase the frequency, electrical conduction and force of cardiac contractions as well as cardiac relaxation; they represent the strongest stimulus for the heart. At the same time they increase the generation of ectopic impulse generation and thereby the risk of arrhythmias. These effects are mediated by $\blacktriangleright$ PKA-mediated phosphorylation of calcium channels (resulting in enhanced calcium influx) as well as of phospholamban, a negative regulator of the sarcoplasmic calcium ATPase $\blacktriangleright$ SERCA (resulting in

enhanced uptake of calcium into the stores of the sarcoplasmic reticulum). A second important localization of β_1 -adrenergic receptors is the cells of the juxtaglomerular apparatus, where they increase the release of renin and thus cause stimulation of the renin-angiotensin system.

β_2 -Adrenergic receptors are located primarily on smooth muscle cells and mediate relaxation. This results in bronchodilatation, relaxation of the uterus and vasodilatation (partially mediated by β_1 -adrenergic receptors). Liver β_2 -adrenergic receptors trigger a protein kinase cascade that results in inhibition of glycogen synthase and activation of phosphorylase and thereby trigger the mobilization of glucose from glycogen stores

β_3 -Adrenergic receptors stimulate lipase and cause the breakdown of triglycerides to fatty acids in fat cells. It is still not clear to what extent the β_2 -subtype participates in this process. Together with the mobilization of glucose from the liver, lipolysis provides the energy sources for the sympathetic “fight-or-flight” reaction.

Polymorphisms have been described for all β -adrenergic receptors; while initial studies, notably of the β_1 -subtype, suggested that these might be predictors of diseases as well as of therapeutic responses, these results have more recently been questioned. Alterations in responsiveness may be limited to certain compounds and a causative role in diseases has not been or remains to be substantiated in larger trials.



β -Adrenergic System. Figure 2 β -Adrenergic signalling in cardiac muscle (predominantly β_1) and smooth muscle (predominantly β_2) cells. Proteins that become more active after activation of β -adrenergic receptors are depicted in grey, those which become less active are depicted in white. Both receptors couple to G_s and lead to activation of adenylyl cyclases, generation of cyclic AMP and activation of protein kinase A (PKA). In *heart muscle cells*, PKA causes phosphorylation of L-type calcium channels (increased Ca^{2+} -influx; relevant site of phosphorylation uncertain), troponin I (TnI; diminishes affinity of troponin C for Ca^{2+} and thus enhances relaxation) and phospholamban (PLB; leads to less inhibition of the sarcoplasmic Ca^{2+} ATPase, SERCA, which pumps Ca^{2+} into the sarcoplasmic stores. This in turn enhances relaxation and enhances Ca^{2+} -release during the next beat) All this leads to more rapid and forceful contraction as well as relaxation. In *smooth muscle cell* the signalling pathways are less clear. PKA-mediated phosphorylation of myosin light chain kinase (MLCK) causes reduced activity of this kinase, which in turn leads to decreased phosphorylation of myosin light chains and, hence, reduced contraction. A second postulated mechanism for relaxation is hyperpolarization via activation of K^+ -channels; the signalling pathway is unclear and might involve coupling of β_2 -receptors to G_i .

Drugs

β -Adrenergic Receptor Agonists

Agonists as well as antagonists of β -adrenergic receptors are used for the treatment of a variety of conditions. β -Adrenergic receptor antagonists belong to the most frequently used classes of drugs.

The main use of β -adrenergic receptor agonists (β -sympathomimetic drugs) is the symptomatic treatment of bronchial asthma. Stimulation of β_2 -adrenergic receptors on smooth muscles produces dilatation of the airways and reduces airway resistance. Although β_2 -adrenergic receptor activation inhibits inflammatory mediator release from mast cells and other inflammatory cells, there is no major effect of these drugs on airway inflammation associated with asthma. β_2 -Adrenergic receptor agonists are the most effective bronchodilators known. In order to reduce unwanted systemic effects (most notably tachycardia and arrhythmia) inhaled β_2 -selective compounds are the drugs of choice; a high first-pass effect helps to reduce systemic effects of the major fraction of the inhaled drug that reaches the gastrointestinal tract. The most frequently used compounds are

fenoterol and salbutamol, which have a rapid onset and a short duration of action. Newer lipophilic compounds such as formoterol and salmeterol have a much longer duration of action (up to 12 h), presumably because they are retained in the plasma membrane after dissociation from the receptor, i.e. they remain in the immediate vicinity and can thus re-associate with the receptor.

β_2 -Adrenergic receptor agonists are also used to treat premature labour by causing uterine relaxation. Fenoterol and ritodrine are frequently used. The effectiveness of long-term tocolysis is controversial, since both desensitization of the receptors and the symptomatic nature of this treatment may limit their effects to 1–2 days according to one large study.

β_2 -Adrenergic receptor agonists, in particular clenbuterol, have been used for their hypertrophic effects on skeletal and also cardiac muscle. They can increase muscle growth in cattle (illegal in many countries) but have also been used by body-builders and athletes as anabolic drugs.

Non-selective β -adrenergic receptor agonists, particularly adrenaline (epinephrine), are used in cardiovascular

emergency situations, most importantly cardiopulmonary resuscitation and anaphylactic shock. They are given to produce stimulation of cardiac electrical activity via β_1 -receptors, inhibition of mast cell mediator release via β_2 -receptors, and bronchodilatation via β_2 -receptors as well as α_1 - and α_2 -receptor-mediated vasoconstriction.

Relatively selective stimulation of β_1 -adrenergic receptors can be achieved with dobutamine. This is a racemic drug of which both isomers activate the β_1 -receptor, and in addition the (–) isomer activates α_1 -receptors whereas the (+) isomer activates β_2 -receptors; the simultaneous activation of α_1 - and β_2 -receptors results in no major net effect on peripheral resistance, and thus the overall cardiovascular effects are mediated by β_1 -stimulation leading to increases in cardiac contractility and output. Dobutamine is used for the short-term treatment of acute cardiac failure and for diagnostic purposes in stress echocardiography.

β -Adrenergic Receptor Antagonists

Clinically used β -adrenergic receptor antagonists (“ β -blockers”) are either β_1 -selective (e.g. bisoprolol, metoprolol, atenolol, betaxolol) or non-selective, i.e. with similar affinity for the β_1 - and the β_2 -subtype (e.g. propranolol, timolol, celiprolol). Many compounds classified as antagonists are in fact inverse agonists, for example metoprolol, bisoprolol, timolol or propranolol; inverse agonism is more pronounced at β_2 - than at β_1 -receptors because the latter possess a lower constitutive activity. Some compounds possess a partial agonist activity (PAA, or intrinsic sympathomimetic activity ISA); examples are pindolol or celiprolol. These drugs produce less bradycardia but may be therapeutically less efficient.

Blockade of β -adrenergic receptors are important in the treatment of many cardiovascular diseases. Supraventricular and ventricular tachycardias are treated by reducing pacemaker currents in the SA-node, slowing AV-conduction and decreasing ectopic impulse generation. This is achieved via reductions in pacemaker currents and Ca^{2+} -currents (class II antiarrhythmic drugs). Blockade of cardiac β_1 -adrenergic receptors (preferentially with β_1 -selective drugs) reduces cardiac frequency, cardiac output, cardiac O_2 -consumption and probably prevents β -adrenergically induced cardiac remodelling. Therefore, they are first-line drugs in the treatment of hypertension, angina, myocardial infarction and cardiac failure. In the latter case, treatment must be initiated with very low doses to prevent acute decompensation. While β -adrenergic receptor antagonists have become the most effective means of treatment in heart failure during the past decade, their first-line use in hypertension has recently been questioned.

Non-selective β -adrenergic receptor antagonists (e.g. propranolol) can suppress tachycardia and tremor in patients with hyperthyroidism or tremor caused by

stress or nervousness. This use is illegal in certain sports (e.g. shooting).

β -Adrenergic receptor antagonists can reduce aqueous humor production in the eye and thereby reduce intraocular pressure. This is why they represent one of the most frequently used class of drugs in glaucoma. Timolol is the best-established compound, followed by levobunolol and others. High concentrations of these compounds are applied to the eye, and it is, therefore, not really clear whether the effects are indeed mediated via specific interactions with β -adrenergic receptors. In recent years, newer drugs acting on prostaglandin or α_2 -adrenergic receptors have become more popular as first-line drugs in glaucoma.

- α -Adrenergic System
- Antiarrhythmic Drugs
- Antihypertensive Drugs
- Catechol-*O*-Methyltransferase and its Inhibitors

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Adrenoceptor

- α -Adrenergic System
- β -Adrenergic System

β -Adrenoceptor System

- β -Adrenergic System

α -Adrenoceptors

► α -Adrenergic System

Adrenomedullin

Human adrenomedullin is a 52-amino acid peptide belonging to the calcitonin/calcitonin gene-related peptide (CGRP)/amylin peptide family. It is synthesized mainly in endothelial cells and elicits vasodilation.

► Calcitonin Gene Related Peptide

Adverse/Unwanted Reactions

All drugs, in addition to their therapeutic effects, have the potential to do harm, i.e. to cause adverse/unwanted reactions (side effects). These may or may not be related to the principal pharmacological action of the drug. Examples of the second category are toxic effects of metabolites of a drug or immunological reactions.

Affective Disorders

Affective (mood) disorders are characterized by changes in mood. The most common manifestation is depression, ranging from mild to severe forms. Psychotic depression is accompanied by hallucinations and illusions. Mania is less common than depression. In bipolar affective disorder, depression alternates with mania.

► Antidepressant Drugs

Affinity

Ligands reside at a point of minimal energy within a binding locus of a protein according to a ratio of the rate

the ligand leaves the surface of the protein (k_{off}) and the rate it approaches the protein surface (k_{on}). This ratio is the equilibrium dissociation constant of the ligand–protein complex (denoted $K_{\text{eq}} = k_{\text{off}}/k_{\text{on}}$) and defines the molar concentration of the ligand in the compartment containing the protein that is bound to 50% of the protein at any one instant. The ‘affinity’ or attraction of the ligand for the protein is the reciprocal of K_{eq} .

► Drug–Receptor Interaction

Age-related Macular Degeneration (AMD)

A disease process characterized by deterioration of the macula of the retina that results in a loss of sharp central vision. AMD is the leading cause of central vision loss in the developed countries today for those over the age of fifty years.

► Tyrosine Kinase Inhibitors

Agonist

Natural or synthetic receptor ligands that induce a conformational change (active conformation) and a signal transduction process upon receptor binding. Agonists may act as typical hormones or neurotransmitters or they may confer paracrine functions, recognize bacterial, viral or other environmental constituents via activating their dedicated receptors.

Ligands that bind to the receptor, but do not exert a maximal cellular reaction when applied at saturating concentrations are referred to as partial agonists. Their remaining activity is termed intrinsic activity ranging between 0% and 100%.

► Drug–Receptor Interaction

► Transmembrane Signaling

► G-protein-coupled Receptors

► Adenosine Receptors

► Chemokine Receptors

► Nuclear Receptors

► Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

► Selective Sex Steroid Receptor Modulators

Agouti-related Protein (AgRP)

The agouti gene encodes a paracrine signalling molecule that antagonizes the effect of melanocyte-stimulating hormone (MSH) at the melanocortin-1 receptor. This effect reduces the synthesis of eumelanin, and is responsible for the agouti hair colour in rodents. Agouti-related protein (AgRP) is similar to the agouti protein (25% identical amino acids), and is an endogenous antagonist of α MSH at the melanocortin-3 and melanocortin-4 receptors. AgRP is a potent orexigen, and down-regulation of AgRP is a major mechanism of the anorexigenic effect of leptin.

► Appetite Control

Ah Receptor

► Arylhydrocarbon Receptor

AIDS

AIDS (acquired immunodeficiency syndrome) is the final stage of disease caused by infection with HIV. In this stage, the virus infection has severely affected the immune system, causing a depletion of CD4⁺ T-helper cells. AIDS is characterized by the manifestation of typical diseases caused by opportunistic infections (Pneumocystis carinii pneumonia, CMV retinitis, candidiasis of the esophagus, cerebral toxoplasmosis), neurological manifestations, cachexia, or certain tumors (Kaposi sarcoma of the skin, B-cell lymphoma).

► Antiviral Drugs

Airway Hyperresponsiveness

Airway hyperresponsiveness is an exaggerated propensity for airways to narrow too easily in response to a wide variety of stimuli. Airway hyperresponsiveness

leads to clinical symptoms of wheezing and dyspnea after exposure to allergens, environmental irritants, viral infections, cold air, or exercise.

► Glucocorticoids
► GABAergic System
► Bronchial Asthma

Airway Surface Liquid

Airway surface liquid (ASL) is the very thin fluid layer (<7 μ M) maintained at the apical membrane of airway epithelia. ASL thickness is maintained by a tight control of fluid reabsorption and/or secretion, mediated by sodium and/or chloride channels.

► Epithelial Na⁺ Channel

AKAPs

AKAPs are cyclic AMP-dependent protein kinase (PKA)-anchoring proteins, a family of about 30 proteins anchoring PKA at subcellular sites in close vicinity to a certain substrate.

► Scaffolding Proteins
► A Kinase Anchoring Proteins (AKAPs)

AKT

Synonyms
PKB

Definition

Akt also known as protein kinase B (PKB) is serine/threonine-specific protein kinase important in mammalian cellular signalling. Akt, originally identified as the oncogene in the transforming retrovirus (AKT8), controls cell survival by inhibiting apoptosis processes. Logically, Akt has been implicated as a major factor in many types of cancer. Akt plays also a crucial role in the insulin signalling pathway.

Akt is activated by binding of plasma membrane phospholipids downstream of insulin receptors, growth and survival factor receptors in a phosphoinositide 3-kinases dependent manner. In humans, there are three genes in the “Akt family”: Akt1, Akt2 and Akt3. Their respective functions are still under investigation.

- ▶ Phospholipid
- ▶ Insulin Receptor
- ▶ Tyrosin Kinases
- ▶ Growth Factors

Alcohol

- ▶ Ethanol

Alcohol Dehydrogenase

Alcohol dehydrogenase is a cytoplasmic enzyme mainly found in the liver, but also in the stomach. The enzyme accomplishes the first step of ethanol metabolism, oxidation to acetaldehyde, which is further metabolized by aldehyde dehydrogenase. Quantitatively, the oxidation of ethanol is more or less independent of the blood concentration and constant with time, i.e. it follows zero-order kinetics (pharmacokinetics). On average, a 70-kg person oxidizes about 10 ml of ethanol per hour.

- ▶ Ethanol

Aldehyde Dehydrogenase

Ethanol is almost entirely metabolized in the liver. The first step, oxidation by ▶ [alcohol dehydrogenase](#), yields acetaldehyde, a reactive and toxic compound. Essentially all of the acetaldehyde is converted to acetate by the liver enzyme aldehyde dehydrogenase. Aldehyde dehydrogenase is inhibited by the drug disulfiram. Given alone, disulfiram is a nontoxic substance. However, ethanol consumption in the presence of

disulfiram causes an extremely unpleasant reaction characterized by flushing, hyperventilation, vomiting, sweating, tachycardia, hypotension, vertigo and marked distress. The altered response to alcohol is the rational basis for the use of disulfiram in the treatment of chronic alcoholism.

- ▶ Ethanol

Aldosterone

Aldosterone is a small hydrophobic molecule and belongs to the class of steroid hormones. Aldosterone is the major mineralocorticoid in the body. It binds to the mineralocorticoid receptor. This receptor belongs to the superfamily of steroid hormone receptors, which are located intracellularly and, upon binding of the agonist, translocate into the cell nucleus, where they regulate the transcription of those genes, which contain the appropriate hormone responsive regulatory elements. In the intestine and particularly in the distal tubules of the kidney, aldosterone increases the expression of proteins which modulate the activity of the sodium–potassium ATPase and the amilorid-sensitive sodium channel. As a consequence, sodium reabsorption and, secondarily, potassium excretion increase.

- ▶ Gluco-Mineralocorticoid Receptors
- ▶ Nuclear Receptors
- ▶ Renin–Angiotensin–Aldosterone System
- ▶ Epithelial Na^+ Channel

Aldosterone Receptor

- ▶ Gluco-Mineralocorticoid Receptors

AlF_4^-

Fluoride forms a tetrahedral ion with aluminum, AlF_4^- , which forms a complex with the $\text{GDP} \cdot \alpha \beta \gamma$ form of heterotrimeric G-proteins. In the case of G_s , the complex $\text{AlF}_4^- \cdot \text{GDP} \cdot \alpha_s \beta \gamma$ behaves much as GTP or the more stable GTP derivatives, $\text{GTP}\gamma\text{S}$ or $\text{GPP}(\text{NH})\text{P}$,

and causes the dissociation of G_s and the subsequent activation of adenylyl cyclase through the complex $AlF_4^- \cdot GDP \cdot \alpha_s \cdot C$. AlF_4^- does not activate small, monomeric GTPases.

- G-proteins
- Adenylyl Cyclases

Alkaloid

Alkaloids are heterocyclic basic compounds and widespread in plants. Many of them have specific targets in organisms. For example, the alkaloids atropine and scopolamine of *Belladonna* are specific antagonists at ► [muscarinic receptors](#).

Alkylating Agents

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Synonyms

Antiproliferative agents; Methylating agents; Chloroethylating agents

Definition

Alkylating agents have the ability to add alkyl groups to many electronegative groups under conditions present in cells. They stop tumor growth by introducing single strand breaks as well as by cross-linking nucleotides in DNA double-helix strands – directly attacking DNA. This makes the strands unable to uncoil and separate. As this is necessary in DNA replication, the cells can no longer divide. Alkylating agents act nonspecifically. Some of them require conversion into active substances *in vivo*.

Mechanism of Action and Clinical Use (Including Side-Effects)

Alkylating agents are DNA-interactive drugs characterized by the formation of covalent DNA adducts. The alkylating agents are the largest class of anti-cancer agents, comprising five subgroups: nitrogen

mustards, alkyl sulfonates, nitrosoureas, ethylenimines and ► [triazines](#) [1].

Several other drugs (e.g., procarbazine, hexamethylmelamine, estramustine, mitomycin C) are thought to act at least in part by alkylation. Alkylating agents are capable of introducing alkyl groups to nucleophilic sites (such as sulfhydryl, amino, phosphate, hydroxyl, carboxyl, and imidazole groups) of other molecules by forming covalent bonds. ► [Alkylation](#) damages the structure and/or function of DNA, RNA and various enzymes. The inhibition of DNA synthesis occurs at drug concentrations that are lower than those required to inhibit RNA and protein synthesis. The degree of DNA alkylation correlates well with the cytotoxicity of these drugs. The interaction with DNA also accounts for the mutagenic and carcinogenic potential of these drugs. The 7-nitrogen (N7) and 6-oxygen (O6) of guanine have been shown to be particularly susceptible to attack by ► [electrophilic compounds](#). There are several possible consequences of N7 guanine alkylation:

- Cross-linkage – bifunctional agents may form covalent bonds with each of two adjacent guanine residues and such inter-strand cross-links will lead to inhibition of DNA replication and transcription. Intra-strand and DNA-protein cross-links may also be formed;
- Mis-pairing of bases – alkylating at N7 changes the O6 of guanine to its enoltautomer, which can then form base pairs with thymine. As a consequence gene miscoding may be induced with adenine-thymine pairs replacing guanine-cytosine. Thus defective proteins are produced;

Alkylating agents, subgroups with examples

Alkylating agent	
Classic (nitrogen mustards)	Mechlorethamine (nitrogen mustard)
	Cyclophosphamide
	Ifosfamide
	Chlorambucil (leukeran)
	Melphalan (alkeran)
Alkyl sulfonates	Busulfan
Nitrosoureas	Carmustine
	Lomustine
	Streptozocin (streptozotocin, zanosar)
Ethylenimines	Thiotepa
Triazines	Dacarbazine
	Procarbazine
Platinum complexes	Cisplatin
	Carboplatin
	Oxaliplatin
Others	Bendamustine

- Depurination – N7 alkylation may cause cleavage of the imidazole ring and excision of the guanine residue, leading to DNA strand breakage.

Nitrogen Mustards

The first successful chemotherapy of malignant tumors was recorded in 1942 when the first cytostatic drug named mechlorethamine (►nitrogen mustard) was used. Later, a large series of nitrogen mustard analogues was synthesized and introduced into the clinical practice (Fig. 1). These analogues are highly reactive compounds that can form covalent bonds with nucleophilic reaction partners in the cell, thus transferring alkyl groups onto nucleic acids and proteins (alkylating reactions). Most of the alkylating agents are bivalent and react by their two chloroethylamine groups. They induce intra-strand and inter-strand cross-links (bridges) within the same DNA strand or between two different DNA strands (so called cross linking). This is the main mode of action of nitrogen mustard and its derivatives. DNA replication is disturbed and eradication or damage of tumor cells is induced. This cytostatic effect is mainly exerted in cells with higher proliferative activity being in the S-phase of the cell cycle when DNA replication (doubling) is performed. Alkylating agents are not phase specific because they act during all

phases of the cell cycle. Hence, although to a lower extent, alkylating agents are able to cause cytotoxic reactions in cells outside of the cell cycle (G0 cells, nondividing cells).

Mechlorethamine (nitrogen mustard): In vivo each chloroethylamine group undergoes intramolecular cyclization with release of a chloride ion. The so formed highly reactive ethylen-immonium derivative alkylates DNA and other biomolecules and causes the cytotoxic effect.

Clinical Use (Including Side-Effects)

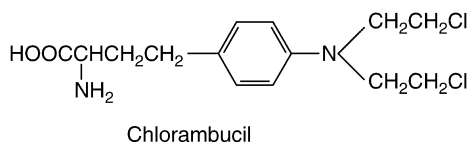
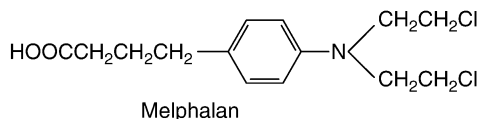
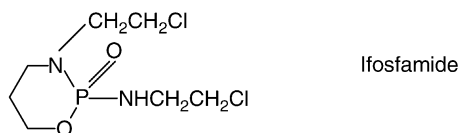
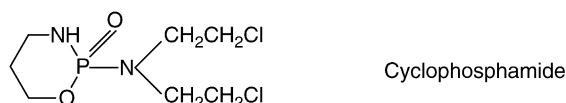
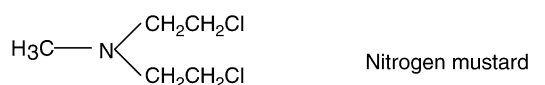
Nitrogen mustard is clinically used for the treatment of lymphomas and some forms of lung cancer. The major indication for mechlorethamine is Hodgkin's disease as a part of the MOPP regimen (mechlorethamine + vincristine (oncovin) + procarbazine + prednisone). The usual dose consists of 6 mg/m² on days 1 and 8. This drug has pronounced hematological toxicity (myelosuppression).

According to a hypothesis launched by Larionov et al in the 1960s, some new nitrogen mustard derivatives were developed. They contain metabolites and heterocyclic structures as carriers of the cytotoxic chloroethylamine groups. By this way the synthesis of *alkylating metabolites* started: melphalan (sarcosine) as L- or DL-phenylalanine derivative; prospidine with a tricyclic piperazine moiety and chlorambucil as butyric acid derivative. It was proven that each alkylating metabolite has its own spectrum of selective antitumor activity.

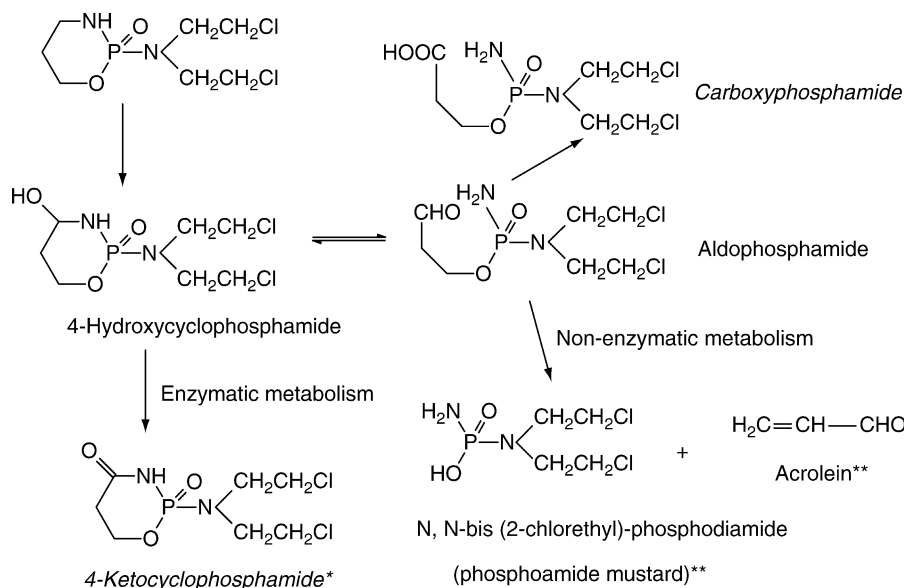
Prospidine influenced human laryngeal tumors. Melphalan showed clinical efficacy in human seminoma and multiple myeloma. It possesses the same general spectrum of antineoplastic activity as other nitrogen mustards do. However, since it does not cause alopecia, melphalan is occasionally substituted for cyclophosphamide in the CMF regimen for breast cancer. Its bone marrow suppression tends to be more prolonged and affects both white cells and platelets. It is widely used in conditioning regimens before stem cell transplantation. Chlorambucil is used for the treatment of chronic lymphocytic leukemia, polycythemia and exerts immunosuppressive activity. It is predominantly used in the treatment of lymphomas and shares teratogenic and carcinogenic properties with the other nitrogen mustards.

German investigators (Brock et al) worked on the creation of alkylating pro-drugs that have cytostatic activity after specific biotransformation in the tumor tissue. Cyclophosphamide (CTX) has well pronounced antitumor activity with the broadest spectrum. It is metabolized to the cytotoxic phosphoamide mustard. In normal tissues with high enzyme level cyclophosphamide is converted to its inactive metabolites (Fig. 2). These differences in biotransformation can explain the relative selectivity of cyclophosphamide towards

Alkylating agents



Alkylating Agents. Figure 1 Chemical structure of some alkylating agents.



Alkylating Agents. Figure 2 Biotransformation of cyclophosphamide – formation of inactive (*) and toxic (**) metabolites.

tumor cells. It is inactive unless metabolized by the liver to 4-hydroxy-cyclophosphamide.

This drug is used for the treatment of breast carcinoma, ovarian tumors, SCLC, multiple myeloma, osteosarcoma and leukemias as a part of combination therapy. High dosages are often curative in Burkitt's lymphoma, a childhood malignancy with very fast growth rate. Oral daily dosages are useful for less aggressive tumors, such as nodular lymphomas, multiple myeloma, and chronic leukemias. Cyclophosphamide can be given orally and as intramuscular, intravenous and intra-arterial injections. Its side effects are typical for alkylating agents and include those caused by damage of cells with high proliferation rate (e.g., hematopoietic cells, gastro-intestinal and epithelial cells). A common toxic effect is the development of hemorrhagic cystitis of the urinary bladder caused by its metabolite acrolein. The risk of developing a carcinoma of the bladder is also increased. Conventional schedules contain therapeutic doses of 100–500 mg/m² within 3–14 days.

Ifosfamide is an isomeric form of cyclophosphamide with analogous mode of action.

Clinical trials showed therapeutic efficacy in a broad spectrum of tumors; these include SCLC, testicular tumors, sarcomas, breast cancer, renal cell cancer, pancreatic tumors and lymphomas. Ifosfamide is less myelosuppressive than cyclophosphamide but is more toxic to the bladder. Therefore it is recommended that ifosfamide is coadministered with the thiol compound mesna to avoid hemorrhagic cystitis and to reduce the risk of developing bladder cancer. Other side effects include neurotoxicity and myelosuppression.

Alkyl Sulfonates

British investigators (Haddow and Timmis 1951) synthesized and studied esters of the methanesulfonic acid. The most active derivative was the dimethylsulfonic ester of 1,4-butanedione, known as busulfan. Busulfan interacts with the thiol groups of proteins and amino acids; some of its metabolites can alkylate the thiols of cysteine, peptides and proteins. Busulfan exerts selective cytotoxic activity in hematopoietic bone marrow cells and inhibits the formation of granulocytes and platelets. It slightly affects the lymphoid tissue.

Clinical Use (Including Side-Effects)

Busulfan was used for the treatment of chronic myeloid leukemia and polycythemia vera.

Nitrosourea Derivatives

All members of the group ►nitrosourea derivatives are derivatives of methyl nitrosourea and have mutagenic and carcinogenic properties. During the past decades nitrosoureas have been widely used for the treatment of solid tumors. Their cytostatic effects are explained by their influence on the biosynthesis of nucleic acids and proteins. They modify the chemical structure of the nucleotides. Alkyl nitrosoureas are highly reactive and alkylate strongly. Bifunctional congeners lead to DNA cross-linking. In addition, they can realize carbamoylation (introduction of the CO-NH₂ group) of biomolecules. Nitrosoureas predominantly affect the synthesis of DNA (replication) rather than synthesis of messenger, ribosomal and transport RNA (transcription). They slightly affect the synthesis of proteins

(translation). Nitrosoureas lead to great disturbances in the cell cycle and proliferation even after single exposure. Nitrosoureas can kill cells in all phases of the cell cycle. These agents undergo hepatic biotransformation to active metabolites such as isocyanates. As lipophilic compounds they easily cross the blood–brain barrier and selectively accumulate in the brain tissue at concentrations 4–5 times higher than that in hepatic and renal tissues. Nitrosoureas have been used for the treatment of gastrointestinal carcinomas. They suppress the growth of lymphoid tumors and metastatic brain tumors. Cross-resistance between nitrosoureas and classic alkylating agents was not observed. They share the feature of causing delayed bone marrow toxicity, which can be cumulative and does appear as long lasting bone marrow suppression.

Streptozotocin is a naturally occurring nitrosourea compound that was isolated from *Streptomyces achromogenes*. Its structure is related to methyl-CCNU. Streptozotocin is similar enough to glucose to be transported into the cell by the glucose transport protein GLUT2, but is not recognized by other glucose transporters. This explains its relative toxicity to beta cells, since these cells have relatively high levels of GLUT2. Streptozotocin is selectively accumulated in islet cells of the pancreas and is used for treating pancreatic islet cell cancer. It acts through methylation of nucleic acids and proteins. In addition, it produces rapid and severe depletion of the pyridine nucleotides nicotinic adenine dinucleotide (NAD) and its reduced form (NADH) in liver and pancreatic islets. Streptozotocin is used for the induction of experimental diabetes in animals.

Clinical Use (Including Side-Effects)

Carmustine is a bicyclohexylnitrosourea (BCNU, Fig. 3) with broad spectrum of antineoplastic activity (e.g., lymphomas, multiple myeloma, sarcomas, brain tumors, gastrointestinal tumors, melanomas). At doses of 80–200 mg/m² it is given i.v. at 6 week's intervals.

Lomustine (2-chlorethyl-3cyclohexyl-1-nitrosourea, CCNU, Fig 3) is a nitrosourea for oral application. It is used for the treatment of Hodgkin's lymphomas, brain tumors and bronchial carcinomas at a dose of 3.5 mg/kg (130 mg/m²) repeated in 6–8 weeks intervals.

Carmustine and lomustine can produce remissions that last 3–6 months in 40–50% of patients with primary

brain tumors. Both drugs also are used as secondary treatment of Hodgkin's disease and in clinical trials with combination chemotherapy of various types of lung cancer.

Semustine (methyl-CCNU) is also suited for oral application but has greater toxicity and is therefore rarely used.

Nitrosoureas have been used in the treatment of non-Hodgkin's lymphomas, multiple myeloma, renal cell carcinoma, and colorectal cancer. They produce severe nausea and vomiting in most patients at 4–6 h after administration. The major site of dose-limiting toxicity is the bone marrow (leukopenia and thrombocytopenia). As alkylating agents, these drugs are mutagenic, teratogenic, and carcinogenic.

Usual dose schedules of streptozotocin involve 500 mg/m² i.v. during five consecutive days. The major toxicity is renal tubular damage. Treatment of metastatic insulinomas may result in the release of insulin from the tumor and subsequent hypoglycemic coma. Less severe toxicities include diarrhea, anemia, and mild alterations in glucose tolerance or liver function tests.

Ethylenimines

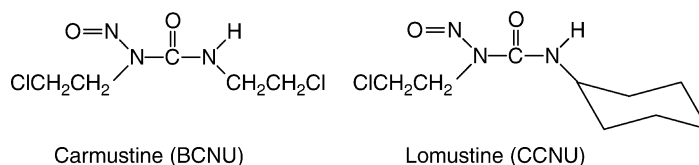
Thiotepa is chemically less reactive than the nitrogen mustards. It has antineoplastic activity against ovarian and breast cancers as well as lymphomas. However, it has been largely supplanted by cyclophosphamide and other nitrogen mustards.

Clinical Use (Including Side-Effects)

It is used by direct instillation into the bladder for multifocal local bladder carcinoma. Nausea and myelosuppression are the major toxicities of thiotepa. It is not a local vesicant and has been safely injected intramuscularly and even intra-theccally.

Triazenes

Dacarbazine is activated by photodecomposition (chemical breakdown caused by radiant energy) and by enzymatic N-demethylation. Formation of a methyl carbonium ion results in methylation of DNA and RNA and inhibition of nucleic acid and protein synthesis. Cells in all phases of the cell cycle are susceptible to dacarbazine. The drug is not appreciably protein bound, and it does not enter the central nervous system.



Alkylating Agents. Figure 3 Chemical structure of some nitrosoureas.

Procarbazine is metabolized in the liver and possibly in tumor cells to yield a variety of free radical and alkylating species.

Temozolomide undergoes spontaneous hydrolysis and decarboxylation at physiological pH value and thereafter a methyl diazonium ion is released. This ion forms DNA adducts within guanine rich DNA sequences. Temozolomide has high bioavailability and is metabolized in the liver.

Clinical Use (Including Side-Effects)

Dacarbazine is the most active compound used for treating metastatic melanoma. It is also combined with anthracyclines and other cytostatics in the treatment of different sarcomas and Hodgkin's disease. Dacarbazine may cause severe nausea and vomiting. Myelosuppression results in leukopenia and thrombocytopenia. Alopecia and transient abnormalities in renal and hepatic function also occur.

Procarbazine causes myelosuppression, hypnotic and other effects on the central nervous system, e.g., vivid nightmares. Also, procarbazine causes a disulfiram like syndrome on ingestion of ethanol.

Temozolomide crosses the blood brain barrier and can be used for the treatment of brain tumors (e.g., *glioblastoma multiforme*). The most common side effects are nausea and vomiting.

Platinum Complexes

The chemical structure of the most frequently used platinum drugs (►platinum complexes) is shown in Fig. 4.

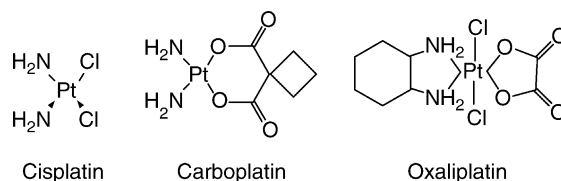
Cisplatin was discovered fortuitously by observing that bacteria present in electrolysis solutions could not divide. It is hypothesized that in the intracellular environment, a chloride is lost and replaced by a water molecule. The resulting species is an efficient bifunctional interactor with DNA, forming platinum-based cross-links similar to that formed by alkylating agents.

Clinical Use (Including Side-Effects)

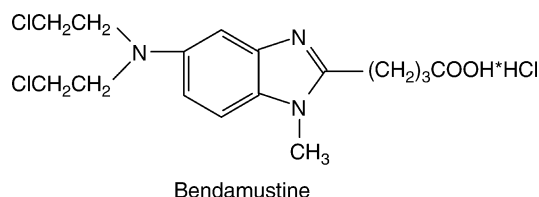
Cisplatin administration requires adequate hydration and forced diuresis to prevent kidney damage. Cisplatin is intensely emetogenic and its use requires adequate antiemetic prophylaxis. Myelosuppression is less evident than with other alkylating agents.

Carboplatin displays less nephro-, oto- and neurotoxicity. However, myelosuppression is more frequent, and as the drug is exclusively cleared through the kidney, adjustment of dose for creatinine clearance must be accomplished.

Oxaliplatin belongs to the group of diaminocyclohexane platinum complexes that can overcome platinum resistance. Its place in the primary and adjuvant treatment of colon cancers is being defined. It is prominently neurotoxic.



Alkylating Agents. Figure 4 Chemical structure of some platinum antineoplastic drugs.



Alkylating Agents. Figure 5 Chemical structure of bendamustine.

Other Alkylating Agents

Bendamustine (Fig. 5) is a representative of the group of bivalent alkylating agents and nitrogen mustard derivatives. It is supposed that bendamustine has a dual mode of action as alkylating agent and antimetabolite due to the presence of a benzimidazole ring and chlorethyl groups in its chemical structure. It is water-soluble and is widely distributed in different tissues. Bendamustine is metabolized in the liver with the formation of monohydroxy and dihydroxy derivatives and some of its metabolites are active antineoplastic compounds. It undergoes renal and biliar excretion.

Clinical Use (Including Side-Effects)

Bendamustine is a useful antineoplastic drug for the treatment of non-Hodgkin's lymphomas, multiple myeloma and as a partner drug in the combination therapy of some solid tumors. The cross-resistance with other alkylating drugs is not complete. Myelosuppression and lymphocytopenia is its main dose-limiting toxicity.

►Antineoplastic Agents

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Alkylation

Alkylation is the transfer of an alkyl group from one molecule to another. The alkyl group may be transferred

as an alkyl carbocation, a free radical or a carbanion (or their equivalents).

► Alkylating Agents

Allele

Allele variant forms of the DNA sequence at a specified locus. For example, alleles at a single-nucleotide polymorphism (SNP) are characterized by the nucleotide that is changing. The combination of two alleles at a locus constitutes a genotype.

► Pharmacogenomics

Allergen

An allergen is usually an inert substance (e.g. pollen, house dust mite faeces) that in some individuals can trigger the generation of an (inappropriate) antigenic response. Mediated by TH2 lymphocytes, it causes B-Lymphocytes to produce IgE. Subsequent exposure of a sensitized individual to the allergen is therefore able to cross-link IgE antibodies on the surface of mast cells and trigger an immune response and histamine release.

- Allergy
- Bronchial Asthma
- Histaminergic System

Allergy

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Synonyms

Hypersensitivity

Definition

The term allergy describes inappropriate immune responses to foreign substances after repeated exposure giving rise to irritant or harmful, and eventually fatal reactions. Its incidence depends on two factors: the occurrence and nature of an agent eliciting immune reactions (allergen) and the reactivity of the immune system (►immune defense). In highly industrialized countries allergies may affect more than 30% of the population caused by poorly understood environmental influences. In addition, a genetically determined predisposition exists to develop an allergy.

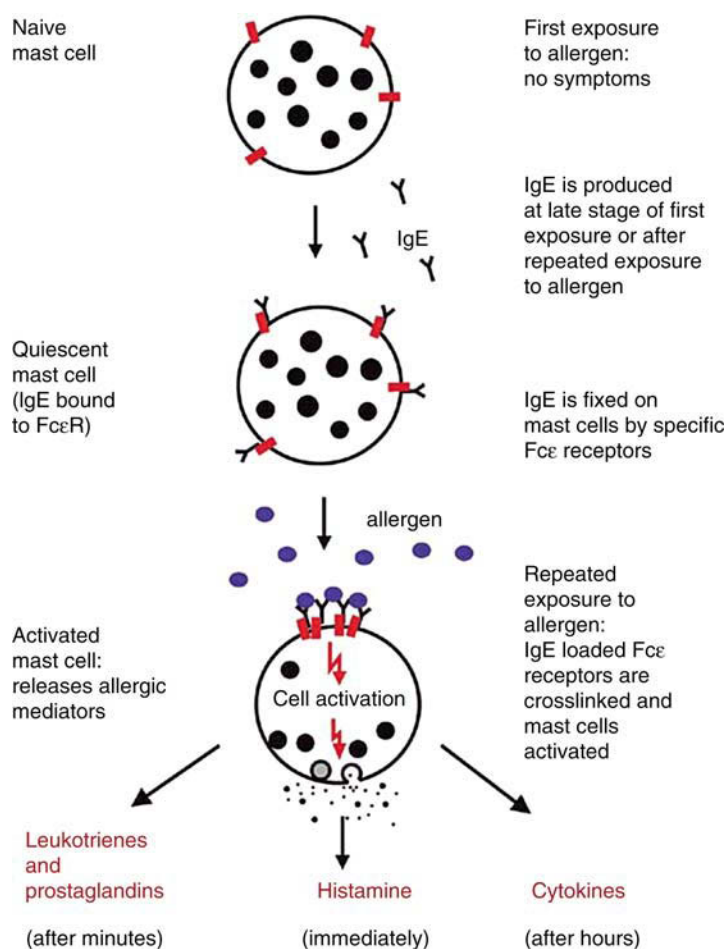
Basic Mechanisms

Currently allergic reactions are classified into four types on the basis of different reaction patterns. Whereas types I–III are dependent on antibodies, the type IV reaction is mediated by cellular immune reactions.

Type I Reactions: Anaphylactic Reaction

This type of allergic reaction is by far the most common one, and may be responsible for more than 80% of all allergies. Often it is used synonymously with allergy.

In some individuals, exposure to an antigen – then termed allergen – leads to the increased production of specific IgE, a subclass of antibodies that physiologically is only synthesized in minute quantities. For this bias of the humoral immune response against an allergen, a subgroup of helper T-lymphocytes, Th-2 cells, plays a key regulatory role by providing the master cytokine interleukin-4 (►immune defense, cytokines). IgE binds with high affinity to receptors (Fcε-receptors) that are present on basophilic granulocytes and most prominently on the closely related mast cells. These cells thus acquire a “borrowed” (as it is, of course, synthesized by B-lymphocytes) allergen specific receptor, which can persist on these cells for long periods, at least several months, perhaps years. If that happens, an individual is “allergic”, i.e. sensitized to an allergen without exhibiting any clinical symptoms and without knowing it. Upon reexposure to this specific allergen, the mast cells (and the other IgE-bearing cells) can immediately recognize the allergen with its IgE antibodies. This results in the crosslinking of the Fcε receptors that in turn triggers activation of the mast cells (or basophils). The consequence is rapid degranulation of preformed vesicles and release of a plethora of mediators into their surrounding (see Fig. 1), the most prominent mediator is histamine which is preformed and stored in vesicles and acts immediately upon release. Within minutes, further mediators like leukotrienes are synthesized. These mediators act in concert on cells in their vicinity which bear the appropriate receptors and thereby cause the clinical symptoms of an immediate allergic reaction. These may be itching (urticaria), local swelling (edema), allergic rhinitis



Allergy. Figure 1 Type I Anaphylactic Reaction: IgE-bearing mast cells are activated by allergens to release mediators of acute allergic reactions.

(“hay fever”), constriction of bronchi (“asthma”), and when occurring in a generalized form, “anaphylactic” shock and eventual death. Activated mast cells also start synthesizing protein mediators, termed cytokines, in a process that requires some time (a few hours). These cytokines initiate an acute inflammatory response that in its late phase is characterized by the infiltration of leukocytes and especially eosinophilic granulocytes.

Type II Reactions: Cytotoxic Reaction

As a physiological response to an antigen, B-lymphocytes initially always secrete antibodies of the IgM class, only in the late stage of the primary response or upon reexposure to the same antigen, B cells switch immunoglobulin classes and produce IgG, IgA, or IgE (►immune defense). In rare situations the antigen, or a metabolite thereof, either alone or bound to a carrier protein, may bind firmly to surfaces of cells. The antigen on the cell surface is now recognized by specific IgG antibodies, and thus the whole cell is labelled as a

“foreign” particle that is consequently – but erroneously – destroyed by the complement system or cellular mechanisms. Type II reactions contribute to autoimmune mechanisms (►autoimmune diseases). They are also responsible for allergic reactions to certain drugs and may induce severe diseases such as drug-induced aplastic anemia or agranulocytosis.

Type III Reactions: Immune Complex Reactions

In the case of the Type III reaction physiologically produced antibodies, predominantly of the IgG subclasses, bind specifically the soluble antigen and form immune complexes. These immune complexes may bind directly to Fcγ receptors or be coated with complement components and thus be opsonized for uptake by phagocytic cells that normally degrade them and thus eliminate them. An “allergic” situation occurs if these immune complexes cannot be ingested appropriately and degraded. Alternatively, and more often, due to a continuous supply of allergen, the phagocytic cell is incapable of coping with the mass of

resulting immune complexes. Thus the phagocytic cells respond to the frustraneous or continuous stimulation of Fc γ receptors by secreting a variety of products into their surrounding. These include catabolic enzymes that degrade unspecifically all available biological macromolecules such as proteins, nucleic acids, carbohydrates, or lipids no matter whether these are foreign or belong to the host, resulting in continuous destruction. It should be noted that this mechanism of damage is identical with that occurring in chronic inflammatory diseases (►inflammation) such as in ►rheumatoid arthritis or nephritis. Typical allergic Type III reactions are pulmonary diseases against inhalative irritants, or “►serum sickness” occurring after administration of high molecular weight proteinaceous drugs, originally animal serum applied during passive vaccination, but also murine monoclonal antibodies or other drugs.

Type IV Reactions: Cellular Reactions

At the time when allergic reactions were classified little was known about cellular reactions, thus it appears appropriate today to divide this reaction type in two subgroups.

Type IVa Reactions: Cellular Cytotoxic Reactions

In this type of reaction an antigen elicits the generation of cytotoxic T-lymphocytes (►immune defense). Cytotoxic T-lymphocytes (Tc) destroy antigen bearing cells by inducing apoptosis. This reaction can be viewed as the cellular counterpart to the humoral Type II reactions. They play an important physiological role in the defense of viruses, and can become allergic reactions under the same conditions as described for Type II reactions.

Type IVb Reactions: Delayed Type Hypersensitivity Reactions

Antigens commonly induce the activation of T-lymphocytes of the T helper type (Th). In the case of Type IV b reactions the predominant responding cell is the Th-1 subtype. By secreting many cytokines, including ►interferon γ , Th-1 lymphocytes recruit and activate granulocytes and monocytic cells to mount an inflammatory response. In that respect the Type IVb reaction can also be viewed as a cellular counterpart of a humoral reaction, specifically of a Type III reaction, being of great importance in chronic inflammatory diseases such as rheumatoid arthritis, and glomerulonephritis, or in autoimmune diseases such as systemic lupus erythematoses. In fact, in these situations both Type III and Type IVb contribute to the chronic inflammatory reaction. With respect to allergy, Type IVb reactions are relevant for contact ekzema, i.e. the chronic response of skin to many irritants including chromate, nickel, cosmetics, fabrics, etc.

Pharmacological Intervention

General

The ideal and single curative treatment of an allergy is to strictly avoid exposure to the responsible allergen(s). This requires to elucidate the causative agent. A battery of diagnostic methods is available to achieve this including measurement of IgE in blood (RAST), various methods of eliciting allergic reactions in the skin (skin testing), and provocation of clinical symptoms (e.g. in food allergy). Unfortunately, many allergens are difficult to avoid in daily life as they occur ubiquitously or, in the case of occupational exposure, would require a change in profession. Thus, in many cases pharmacological intervention may be necessary to improve the health of the allergic patient.

Type II, III, and IV allergic reactions are variants of physiologic defense mechanisms only relevant in special situations, which follow a common pathologic pattern. In general, treatment of these forms require anti-inflammatory (►inflammation) or immunosuppressive strategies (►immunosuppression). Therefore, only therapy of Type I reactions will be described here.

Therapy of Type I Reactions

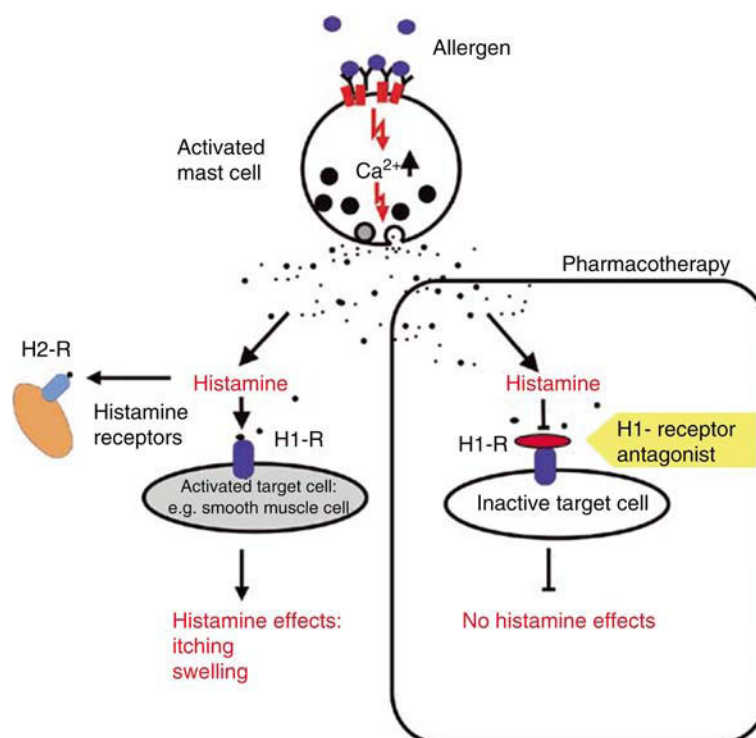
(Rush) Immunotherapy (Hyposensitization)

The inappropriate production of IgE to an allergen is caused by a Th-2 preponderance upon exposure to the allergen. Immunotherapy aims to influence the undesired Th-2 immune response and shift it to a Th-1 answer. It was found empirically and consists of the application of increasing doses of the allergen within a few days, starting with a very low, clinically inapparent, dose, and ending with a dose close to or above the one which is to be expected in a natural situation (e.g. after a bee sting). The high dose usually is applied at monthly intervals for up to three years or even longer. An absolute indication for immunotherapy are allergies to bee or wasp poison, which may result in an anaphylactic shock and may be fatal. At least partial relief may be achieved by immunotherapy in patients with allergies against defined pollen, but mostly fails with complex mixtures of allergens such as proteins of pets (epithelia, hair) or proteins in the faeces of mites (house dust allergy). With the availability of modern molecular biology and the achievements of recombinant DNA technology the identification of the responsible structures of allergens and their production in defined quality and quantities may increase the rate of success also with complex allergens or mixture of allergens in future.

Pharmacotherapy

Histamine H-1 Receptor Antagonists

Histamine H-1 receptor antagonists compete with the binding of histamine to its Type I receptors which are predominantly located in cells of the vasculature, and



Allergy. Figure 2 Histamine H-1 receptor antagonists inhibit response of target cells to histamine and relieve hay fever-like symptoms.

thus block its action (Fig. 2). H-1 receptor antagonists are effective when symptoms occur which involve peripheral blood vessels – around which the majority of mast cells are located – such as urticaria, allergic rhinitis (“hay fever”), or conjunctivitis. As histamine receptors are also present in the brain, their blockade influences the ability to focus and may result in sleepiness. However, modern second generation H-1 receptor antagonists do not cross the blood–brain barrier and thus do not show this undesired sedative side effect (examples: loratadin, fexofenadin, cetirizin).

Leukotriene Antagonists

Leukotrienes are rapidly produced and released during a Type I reaction (Fig. 3). They are responsible for a massive bronchoconstriction in allergic bronchial asthma and attract leukocytes, thus being proinflammatory. Consequently, antagonists of the LTC receptor have been proven useful in the therapy of ▶ bronchial asthma, often in combination with bronchodilators (example: montelukast).

Cromones

Cromones suppress the release of mediators from mast cells by a mechanism that is not known (Fig. 4). In order to achieve the complete suppressive effect, cromones have to be given prophylactically several days to weeks before exposure to seasonal allergens can be expected,

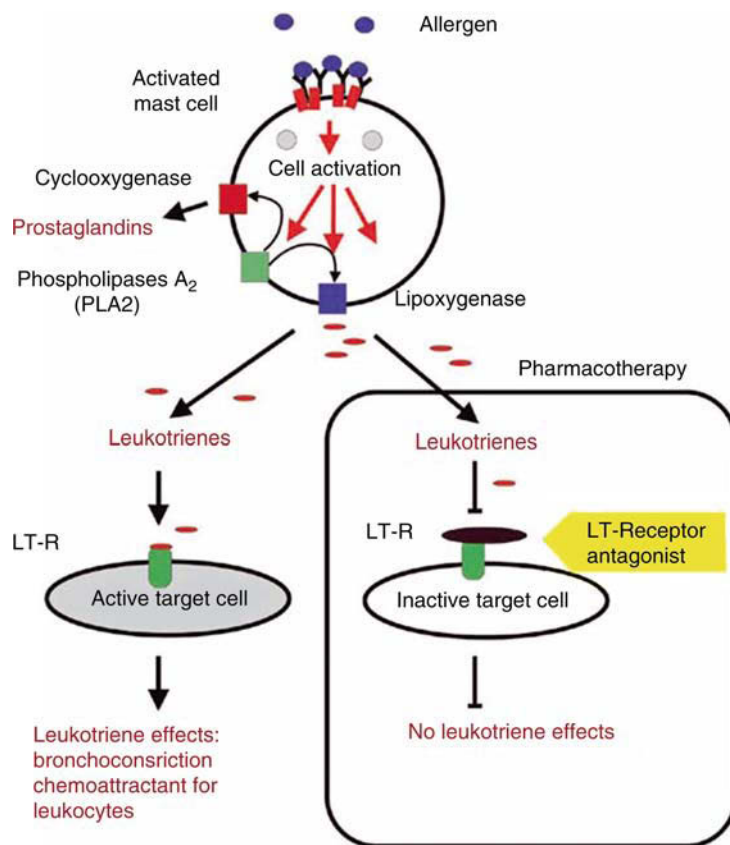
emphasizing the importance of warning systems or calendars (e.g. for pollen) as means for initiating therapy. Cromones also are effective in ongoing allergic responses, but then a few days are required to see benefits for the patient. Cromones are practically insoluble and thus are not absorbed beyond the top layers of tissues. This has the advantage that no systemic side effects occur, on the other hand cromones only act locally and must be applied at the site of action wanted. Cromones have beneficial effects in allergic rhinitis (nose drops or spray), conjunctivitis or bronchial asthma (inhalable preparations) (examples: disodium cromoglycate or nedocromil).

Glucocorticoids

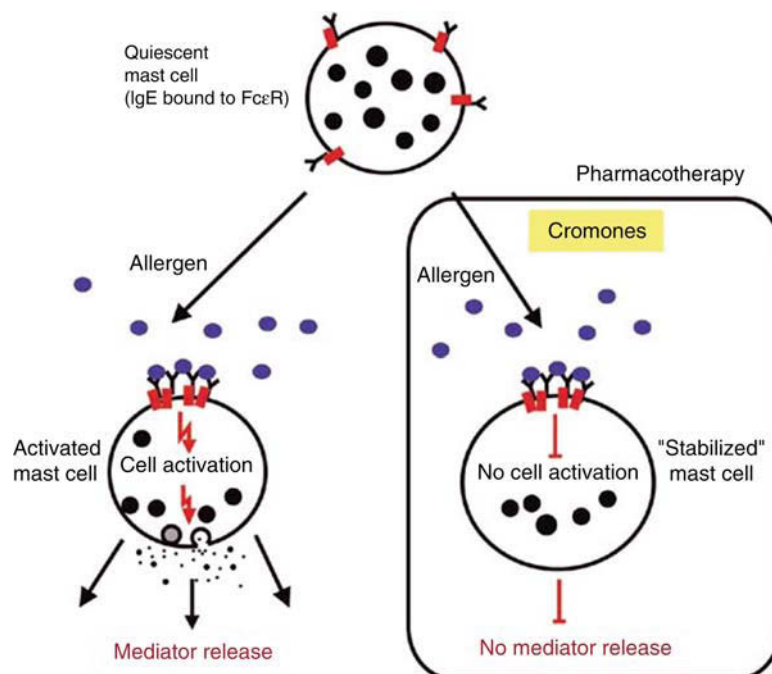
Topically Applied Glucocorticoids – “Inhalable”

Glucocorticoids

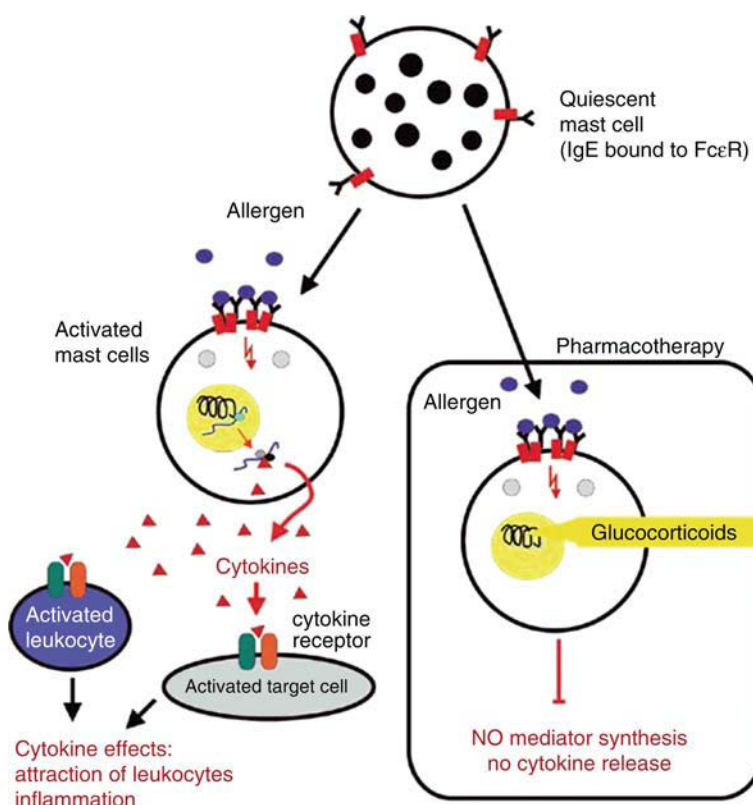
Glucocorticoids are very effective anti-inflammatory drugs. In Type I allergy they affect several different target cells, the most important being the mast cells and infiltrating T-lymphocytes. In general their action is immunosuppressive (▶ immunosuppression). On mast cells, glucocorticoids mainly affect the synthesis and release of mediators such as the arachidonic acid metabolites and most prominently the cytokines. The molecular mechanism of glucocorticoid action is complex and can be summarized as a regulatory effect on gene induction and expression (Fig. 5).



Allergy. Figure 3 Leukotriene LT receptor antagonists inhibit the response of target cells to leukotrienes and relieve symptoms of allergic asthma bronchiale.



Allergy. Figure 4 Cromones "stabilize" mast cells.



Allergy. Figure 5 Glucocorticoids regulate gene expression, resulting in a decrease of cytokine and mediator release.

Of all glucocorticoids applied to the upper respiratory tract (nose, bronchi) more than 80% may be swallowed and finally absorbed by the gastrointestinal tract. This fraction reaches the circulation after an initial first passage through the liver. “Modern” glucocorticoids for inhalation are chemically modified in a way that they are completely inactivated metabolically by the liver. Thus inhalable glucocorticoids in therapeutic doses are effective in the respiratory tract, but do not give rise to systemic side effects (►glucocorticoids). They play an important role in the long term treatment of ►bronchial asthma. They also have beneficial effects in allergic rhinitis (“hay fever”), especially in seasonal forms (example: beclomethason, budesonid).

Glucocorticoid Ointments

Glucocorticoid ointments are used to treat allergic skin reactions locally. They should be applied only for limited periods to avoid trophic damage to the skin such as thinning (paper skin).

Systematically Applied Glucocorticoids

Because of their considerable side effects – which depend on dose and, even more relevant, on the duration of application – systemically applied glucocorticoids are only used in serious allergic diseases.

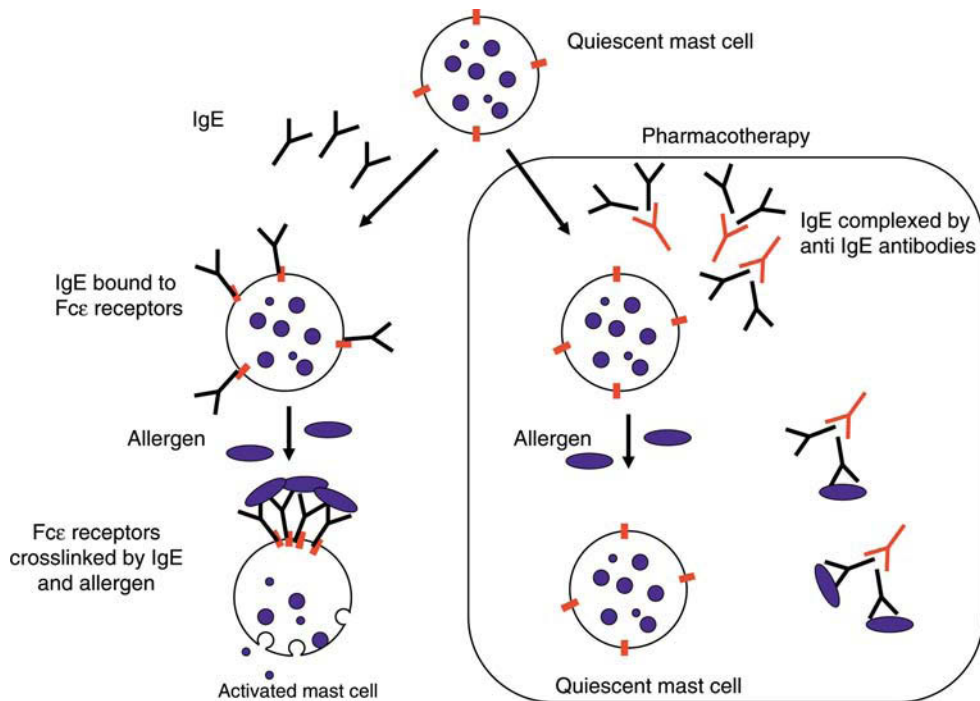
This includes ►bronchial asthma, autoimmune, and chronic inflammatory diseases.

Anti-IgE Antibodies

A modern strategy of pharmacological intervention aims at the neutralization of immunoglobulin E before it binds to the Fcε receptors on the mast cells. This is achieved by applying a monoclonal antibody recognizing the Fc part of human IgE antibodies irrespective of their antigen specificity. By this means IgE cannot bind to the Fcε receptor on mast cells, basophils, and dendritic cells. Thus activation of these cells does not take place even in the presence of allergen, because the allergen will bind to the IgE molecules kept in solution by the neutralizing antibody, and the mast cells remain quiescent (Fig. 6). The humanized monoclonal antibody of the IgG1 type is indicated in patients with moderate and severe asthma if it cannot be managed with glucocorticoids (Omalizumab, Xolair®).

Anaphylactic Shock

The most serious acute Type I reaction is the generalized reaction, the anaphylactic shock. Anaphylactic shock results from a generalized release of mediators from mast cells and basophils. The clinical symptoms are manifested predominantly in



Allergy. Figure 6 Anti IgE antibodies prevent IgE from binding to their receptors on mast cells, and thus from releasing allergic mediators.

1. Circulation: Leakage of fluid from the vasculature into the surrounding tissue causes edema, drop in blood pressure and finally hemodynamic shock.
2. Heart: Histamine (and also other mediators) induce arrhythmias which can be fatal.
3. Respiratory tract: all symptoms associated with allergy can occur, starting from profuse rhinitis to severe asthma and suffocation.
4. Gastrointestinal system: cramps and diarrhea.
5. Skin: generalized urticaria and erythema.

Treatment

The fate of the patient largely depends on the first 30 min of an anaphylactic shock reaction. Thus persons with a known history of hypersensitivity reactions towards bee or wasp poison should always carry an emergency set during the insect season (see below).

1. Intravenous infusion of epinephrin: 0.1–0.5 mg epinephrin dissolved in plasma replacement, this can be repeated after 5 min.
2. Plasma replacement (any): this should also be used as a continuous access to the intravenous blood.
3. Glucocorticoids: 300 mg to 1 g as bolus
4. Histamine H-1 receptor antagonist

Emergency Set Contains:

1. a ready to use epinephrine solution in a special syringe allowing sequential application in two doses

2. readily resorptive glucocorticoid solution (orally)
3. readily resorptive histamine H-1 receptor antagonist (**cave:** sedation!!)
4. (if available: inhalable epinephrine)

► Immune Defense

► Inflammation

► Immunosuppressive Agents

► Humanized Monoclonal Antibodies

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Allodynia

The sensation of pain, following injury or disease, in response to a previously non-noxious stimulus is termed 'allodynia'. Tactile allodynia is caused by

recruitment of low-threshold (non-nociceptive) sensory fibres (A β) in nociceptive pathways.

- Pain and Nociception
- Galanin Receptors

Alloimmunity

Alloimmunity is the immune response mounted by a host on the basis of differences in major histocompatibility antigens expressed on the surface of a donor cell from the same species as the host.

- Immune Defense

Allostatic State

A state of chronic deviation of a regulatory system from its normal (homeostatic) operating level is defined as an allostatic state. In the context of drug addiction this term has been introduced by George Koob and Michel Le Moal and represents a chronic deviation of reward set point by dysregulation of reward circuits and brain stress systems that provide a negative motivational state that drives addictive behavior.

- Drug Addiction/Dependence

Allosteric Modulators

Unlike competitive antagonists that bind to the same domain on the receptor as the agonist, allosteric modulators bind to their own site on the receptor and produce an effect on agonism through a protein conformational change. Allosteric modulators can affect the affinity of the receptor for the agonist or simply the responsiveness of the receptor to the agonist. A hallmark of allosteric interaction is that the effect reaches a maximal asymptote corresponding to saturation of the allosteric sites on the receptor. For example, an allosteric modulator may produce a maximal 10-fold decrease in the affinity of the receptor for the agonist upon saturation of the allosteric sites on the receptor.

- Drug–Receptor Interaction
- Adenosine Receptors

- Metabotropic Glutamate Receptors
- Benzodiazepines

Allylamines

- Antifungal Drugs

ALS

- Amyotrophic Lateral Sclerosis

Alternative Splicing

Alternative splicing is the process occurring when eukaryotic pre-mRNA, which includes several introns and exons, is transcribed from one gene to undergo distinct cutting and pasting processes yielding different mature mRNAs. It is an important cellular mechanism that leads to temporal and tissue-specific expression of unique mRNA products from a single gene. It thereby increases protein diversity by allowing multiple, sometimes functionally distinct, proteins to be encoded by the same gene.

- Ca²⁺ Channel Blockers
- Cholinesterases

Alzheimer's Disease

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Synonyms

Morbus Alzheimer (most common form of dementia)

Definition

Alzheimer's disease (AD) is a progressive, degenerative and irreversible neurodegenerative disorder of the brain. The greatest risk factor for developing AD is increasing age. The likelihood of developing AD doubles almost every five years after age 65, reaching nearly 50 percent risk after age 85. ► **Familial Alzheimer's disease (FAD)** is a hereditary form of the disease that occurs earlier in life with an onset before the age of 65 and accounting for 5–10% of total AD cases. The majority of cases is sporadic without a clear inheritance pattern and occurs after age 65. So far, there is no cure for the impaired memory and the loss of mental abilities affecting normal activities of daily living. Cognitive tests and exclusion of other diseases are used to diagnose AD with a restricted accuracy in living patients. A confirmation of the diagnosis is only possible by autopsy. The key pathological hallmarks of AD are the extracellular ► **amyloid** plaques and intracellular neurofibrillary tangles and its constituent, the tau microtubule-associated protein. The principle component of plaques is the amyloid beta peptide ($A\beta$). The $A\beta$ peptide is derived from the amyloid precursor protein (APP) by two consecutive proteolytic cleavage events. Aggregates of the $A\beta$ peptide are ultimately believed to cause the neurodegeneration that leads to AD.

Basic Mechanisms

The Amyloid Hypothesis

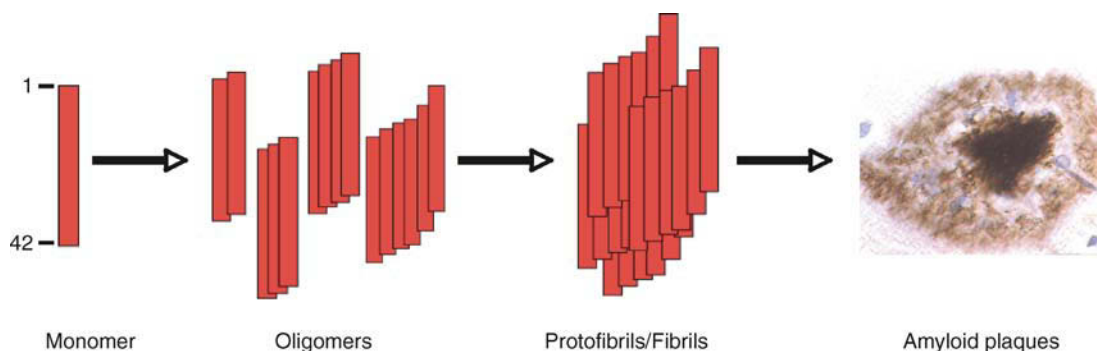
According to the “amyloid hypothesis,” the $A\beta$ peptide plays a critical role in the pathogenesis of Alzheimer's disease [1]. Major forms of $A\beta$ produced encompass 38, 40 or 42 residues. $A\beta_{42}$ is more prone to aggregation than $A\beta_{40}$ and in animal models an increased $A\beta_{42}/A\beta_{40}$ ratio results in amyloid plaque pathology even when total $A\beta$ levels are reduced [4]. The generation of $A\beta$ is a normal process and $A\beta$ is present in the brains and body fluids of humans throughout life. Neuronal

injury appears to result from an ordered self-association of $A\beta$ monomers or dimers before plaque formation can be observed. A loss of neurons was observed in brains of transgenic mice expressing human mutant amyloid precursor protein at sites of $A\beta$ aggregation but, most importantly, was also clearly observed in brain areas distant from plaques. This finding together with the correlation found between levels of soluble $A\beta$ oligomers and the severity of synaptic loss, led to the conclusion that aggregation of $A\beta$ into oligomers is essential for toxicity. $A\beta$ monomers can spontaneously aggregate into oligomers or protofibrils on the pathway to fibril formation (Fig. 1). It seems that oligomerization is an intermediate step in the formation of protofibrils or the end point of an alternative non-fibrillar assembly process prior to plaques or tangle pathology [3].

Oligomeric forms of $A\beta$ either derived from synthetic $A\beta$ or purified from $A\beta$ -containing cell culture medium could be shown to trigger hippocampal synapse loss and are thus suspected to be the effectors of synaptic dysfunction in AD. Based on these findings and findings obtained from APP transgenic mice and human brain tissue the amyloid hypothesis of AD pathogenesis has been modified. Rather than extracellular plaque $A\beta$ causing toxicity, neuronal $A\beta_{42}$ accumulation may play a direct role in causing neuronal dysfunction, neuronal death and dementia caused by oligomer formation of $A\beta_{42}$ inside of the cells.

$A\beta$ is Derived from the Amyloid Precursor Protein (APP)

APP is a type-I transmembrane protein that is part of an evolutionarily conserved protein family, including the amyloid precursor-like proteins 1 (APLP1) and 2 (APLP2). APP and APLPs are functionally redundant and form homo- and hetero-oligomers. The absence of the $A\beta$ sequence in the APLPs underlines the importance of APP that can only give rise to the $A\beta$ species. Decreasing the formation of soluble $A\beta$



Alzheimer's Disease. Figure 1 $A\beta$ monomers can self-associate to form dimers, trimers and higher oligomers. Globular structures of synthetic $A\beta_{42}$ are known as $A\beta$ -derived diffusible ligands (ADDLs) (3–12-mers of $A\beta$). These structures are similar to the smallest protofibrils and represent the earliest macromolecular assembly of synthetic $A\beta$. The characteristic amyloid fiber exhibits a high beta-sheet content and is derived in vitro by a nucleation-dependent self-association and an associated conformational transition from random to beta-sheet conformation of the $A\beta$ molecule. Intermediate protofibrils in turn self-associate to form mature fibers.

monomers and dimers to prevent the generation of cytotoxic oligomers should prove an effective strategy against AD.

APP undergoes proteolytic processing by several ►secretases. First, the bulk of the ectodomain needs to be removed by membrane-bound α - or β -secretases leading to secreted forms of APP and membrane-bound C-terminal fragments α -CTF or β -CTF, respectively. Regulated intramembrane proteolysis (RIP) of the β -CTF by γ -secretase occurs only after ectodomain shedding and releases the A β peptide from the membrane (Fig. 2).

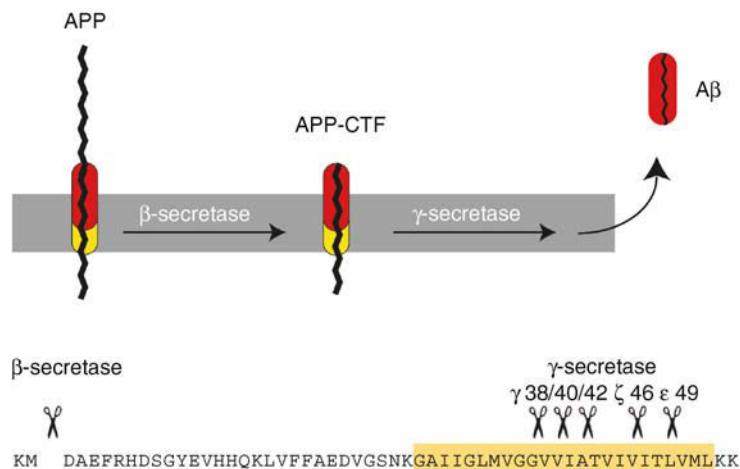
The γ -secretase has been reported to cleave at variable sites thus generating A β peptides of varying lengths with mainly 37–43 residues. An explanation for the multiple cleavages of γ -secretase was provided indicating a sequential proteolytic cleavage mechanism to release A β [2]. Accordingly, the first cut occurs at the cytoplasmic edge of the transmembrane sequence (TMS) at the ϵ -site, i.e. residue 49 or 48 of β -CTF. The products A β 49/A β 48 remain membrane-bound and are further processed in a sequential action mode into A β 46/A β 45, i.e. the ζ -site. A β 46 is further processed into A β 43 and finally A β 40 whereas A β 45 is the direct precursor of A β 42. The underlying mechanism for the regulation is uncertain but alterations in A β production may be reflected by the dynamic nature of the substrate and/or altered enzyme/substrate interactions. The current view is that the generation of A β 42 and A β 38 levels can be regulated by the strength of homophilic interactions within the APP TMS itself leading to the generation of the shorter species A β 37, A β 35 and A β 34 if the interaction is attenuated. Any events stabilizing dimerization would increase the production of A β 42 and vice versa. It could be considered that AD risk factors like

high cholesterol levels or disturbed lipid homeostasis, oxidative stress, disturbed metal homeostasis or familial AD mutations affect the dimer stability of β -CTF and thus influences A β production. Compounds targeting the dimerization of the APP TMS and interfering with the interaction motif of two γ -secretase substrate molecules may be useful as future therapeutics to prevent generation of A β 42.

Pharmacological Intervention

The current treatments cannot cure the disease but are suspected to offer patients modest improvement in some symptoms. For treating the cognitive symptoms of AD, a class of drugs known as cholinesterase inhibitors, such as Galantamin, Rivastigmin, Donepezil and Tacrin, act in delaying the breakdown of acetylcholine that facilitates normal functioning of nerve cells. Another approved agent, Memantine, is an uncompetitive NMDA antagonist which prevents the excessive binding of glutamate to the receptor. Other examples used to treat behavioral and psychiatric symptoms of AD include antidepressant medications for low mood and irritability as well as anxiolytics and antipsychotic medications.

Drugs in clinical development that directly target the A β pathway are at an early stage. Inhibitors of β - and γ -secretases that can lower the A β production have entered clinical phase trials with β -secretase inhibitors being years behind the development of γ -secretase inhibitors. Functional γ -secretase inhibitors have been shown to reduce the rate of A β formation *in vitro* and *in vivo*. The reduction of A β monomer levels could prevent oligomer formation and subsequent synaptotoxicity. Numerous anti-amyloid approaches to



Alzheimer's Disease. Figure 2 A β is derived from the APP by the sequential action of proteolytic activities exerted by β - and γ -secretases. APP-CTF is (C99) produced after cleavage of the APP by β -secretase and represents the substrate of the γ -secretase. The yellow box marks membrane embedded amino acid residues of A β peptide. Scissors represent the main cleavage sites of β - and γ -secretase, e.g. the ζ -, ϵ -, and γ -cleavages at positions 49, 46, 42, 40 and 38.

disassemble, neutralize or degrade A β oligomers are under development. For the latter, an up-regulation of enzymes involved in A β degradation, such as over-expression of neprilysin (NEP) or the insulin degrading enzyme (IDE) led to the desired effects. A β levels in APP transgenic animals were found decreased and plaque burden was reduced. Alternatively, inhibitors of A β aggregation have been tested *in vitro* for two different purposes: first, to stabilize the monomer and then to shift these molecules into the normal degradation pathway and second, to bind to A β oligomers and to disrupt these structures. The compound tramiprosate is a sulfated glycosaminoglycan mimetic, which is suggested to preferentially bind to soluble A β and can inhibit plaque formation in APP transgenic mice. Other compounds such as AZD-103 (scyllo-cyclohexane-hexol) were shown to disassemble large A β oligomers and to neutralize A β dimers and trimers. Similarly, it has been shown that anti-A β antibodies hold a great promise for elucidating the A β clearance by redistributing A β from the brain to the systemic circulation. New therapeutic approaches based on the assumption that the APP pathway underlies the cause of the disease will have more and more to consider structural and functional information on the biological roles of APP and APLPs that may prove useful for novel strategies against this devastating disorder. Thus, compounds targeting the oligomerization mediated by the APP TMS and interfering with the interaction of two γ -secretase substrate molecules may be useful as therapeutics to prevent generation of A β 42.

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Ames Test

The Ames test measures the reversion from mutant to wild type form (back-mutation) in a culture of *Salmonella*. The test is used to screen large numbers of compounds for their potential mutagenicity.

Amiloride

Prototypic inhibitor of the family of Na/H exchangers and of epithelial Na⁺ channels.

- Epithelial Na⁺ Channel
- Na⁺/H⁺ Exchangers

Amiloride-sensitive Na⁺ Channel

The amiloride-sensitive Na⁺ channel (ENaC) is a cell membrane glycoprotein selective for sodium ions, which is composed of three subunits (α , β and γ). Gating of sodium is inhibited by the diuretic amiloride.

- Epithelial Na⁺ Channels
- Na⁺/H⁺ Exchangers
- Endothelins

γ -Aminobutyric Acid (GABA)

- GABAergic System

Aminoglycosides

- Ribosomal Protein Synthesis Inhibitors

Aminopeptidase

An exopeptidase that sequentially releases an amino acid from the N-terminus of a protein or peptide. Examples include cystinyl aminopeptidase (MEROPS M01.011), which removes a terminal cysteine from the biologically important peptides oxytocin and vasopressin, and methionyl aminopeptidase (M24.001), which removes the initiating methionine from cytosolic

proteins as they are being synthesized. Aminopeptidases are included in Enzyme Nomenclature subclass 3.4.11.

►Non-viral Peptidases

AMP, Cyclic

- Cyclic Adenosine Monophosphate
- Adenylyl Cyclases

AMP-activated Protein Kinase

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Synonyms

AMP-activated protein kinase, AMPK; SNF1 complex (fungi); SNF1-related kinase-1 (higher plants)

Definition

The AMP-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that acts as a sensor of cellular energy status [1, 2, 3]. It is activated, via mechanisms described below, by an increase in the cellular AMP:ATP ratio. Because all eukaryotic cells express a very active adenylate kinase, which maintains the reaction $2\text{ADP} \leftrightarrow \text{ATP} + \text{AMP}$ close to equilibrium at all times, any increase in the ADP:ATP is amplified into a much larger increase in the AMP:ATP ratio. This can happen due to a metabolic stress that interferes with ATP production (e.g. hypoxia, glucose deprivation), or that increases ATP consumption (e.g. muscle contraction). Once activated by a fall in cellular energy status or other stimulus, AMPK switches on ATP-generating catabolic pathways, while switching off ATP-consuming processes to restore energy status. The system is the primary target of the ►biguanide drugs (e.g. ►metformin), and is both a direct and indirect target of the ►thiazolidinediones (e.g. ►rosiglitazone, ►pioglitazone), all of which are used as insulin-sensitizing drugs in the treatment of type 2 diabetes.

Basic Characteristics

AMP-activated protein kinases are heterotrimeric complexes comprised of catalytic α subunits and regulatory β and γ subunits (Table 1). Each subunit is encoded by at least two genes, some of which can also be subject to alternate splicing, leading to a diverse array of possible heterotrimeric combinations.

The α subunits, for which two isoforms exist in mammals ($\alpha 1$, $\alpha 2$), contain conventional protein serine/threonine kinase domains at the N-terminus, with a threonine residue in the activation loop (Thr-172) that must be phosphorylated by upstream kinases (see below) before the kinase is active. The kinase domain is followed by an autoinhibitory domain, whose effect is somehow relieved by interaction with the other subunits. The C-terminal domain of the α subunit is required for the formation of a complex with the C-terminal domain of the β subunit, which in turn mediates binding to the γ subunit. The $\alpha 1$ and $\alpha 2$ catalytic subunit isoforms are widely distributed, although $\alpha 2$ is most abundant in muscle and may be absent in cells of the endothelial/hemopoietic lineage.

The β subunits, for which two isoforms exist in mammals ($\beta 1$, $\beta 2$), contain a central glycogen-binding domain. This domain, related to noncatalytic domains found in enzymes that metabolize the $\alpha 1 \rightarrow 6$ branch points in $\alpha 1 \rightarrow 4$ -linked glucans, such as starch and glycogen, causes the AMPK complex to bind to glycogen, although the physiological role of this remains unclear. The β subunits also contain a C-terminal domain that is responsible for the association with the α and γ subunits. The two β subunit isoforms ($\beta 1$ and $\beta 2$) are widely distributed, although $\beta 2$ is most abundant in muscle and may be low or absent in some cell types.

The γ subunits, for which three isoforms exist in mammals ($\gamma 1$, $\gamma 2$, $\gamma 3$), contain variable N-terminal regions followed by four tandem repeats of a 60 residue sequence known as a CBS motif. CBS motifs, named after the enzyme cystathionine β -synthase in which they are also found, occur in around 20 proteins in the human genome and always occur in tandem pairs. A pair of CBS motifs form a stable domain known as a Bateman domain, the function of which appears to be the binding of regulatory adenosine-containing ligands such as AMP, ATP, or S-adenosylmethionine (the latter being an activating ligand for cystathionine β -synthase). Bateman domains have central clefts formed by two hydrophobic β -sheets, with the adenosine moiety of the ligand binding within this cleft. The three AMPK γ subunits are unusual, in that they contain two Bateman domains (i.e. four CBS motifs), each of which binds one molecule of the activating nucleotide, AMP, or the inhibitory nucleotide, ATP. Thus, two molecules of nucleotide bind to each γ subunit, with binding being highly cooperative. The γ subunits also contain a short

AMP-activated Protein Kinase. Table 1 Information about subunit isoforms of AMP-activated protein kinase. Data refer to the full-length forms of the human isoforms. The $\gamma 2$ and $\gamma 3$ isoforms also exist as splice variants that are N-terminal truncations, with lower molecular mass and number of amino acids (38 kDa and 328 amino acids for the short form of $\gamma 2$, 52 kDa and 464 amino acids for the short form of $\gamma 3$). Other splice variants may also exist

Isoform	Mass (kDa)	Gene name	Amino acids	Domains	Domain location (approx.)	Domain function	Site of major expression	Chromosome location
$\alpha 1$	63	PRKAA1	550	Kinase domain	1–270	Catalytic	Ubiquitous?	5p11–p14
				Autoinhibitory domain	290–335	Regulatory		
				C-terminal domain	393–550	β subunit binding		
$\alpha 2$	62	PRKAA2	552	Kinase domain	1–268	Catalytic	Muscle, liver	1p31
				Autoinhibitory domain	288–333	Regulatory		
				C-terminal domain	397–552	β subunit binding		
$\beta 1$	30	PRKAB1	270	Glycogen binding domain	72–151	Glycogen binding	Ubiquitous?	12q24.1–24.3
				C-terminal domain	179–270	$\alpha\gamma$ binding		
$\beta 2$	30	PRKAB2	272	Glycogen binding domain	72–154	Glycogen binding	Muscle	1q21.1
				C-terminal domain	181–272	$\alpha\gamma$ binding		
$\gamma 1$	38	PRKAG1	331	Association domain	17–42	β subunit binding	Ubiquitous?	12q12–q14
				Bateman domain 1	43–178	AMP/ATP binding		
				Bateman domain 2	199–331	AMP/ATP binding		
$\gamma 2$	63	PRKAG2	569	N-terminal domain	1–247	Targetting?	Muscle?	7q36
				association domain	248–274	β subunit binding		
				Bateman domain 1	275–410	AMP/ATP binding		
				Bateman domain 2	431–556	AMP/ATP binding		
$\gamma 3$	54	PRKAG3	328	N-terminal domain	1–171	Targetting?	Muscle	2q35
				Association domain	172–197	β subunit binding		
				Bateman domain 1	198–333	AMP/ATP binding		
				Bateman domain 2	358–454	AMP/ATP binding		

conserved sequence of about 25 residues immediately N-terminal to the first CBS motif, which appears to be involved in the interaction with the β subunit. The function of the variable N-terminal domains present in the $\gamma 2$ and $\gamma 3$ isoforms (some or all of which can be

removed by RNA splicing) are not known, although one possibility is that they are involved in subcellular targeting of the complex. The $\gamma 1$ isoform is the major isoform in most cell types and appears to be universally distributed. The $\gamma 2$ isoform is most abundant in skeletal

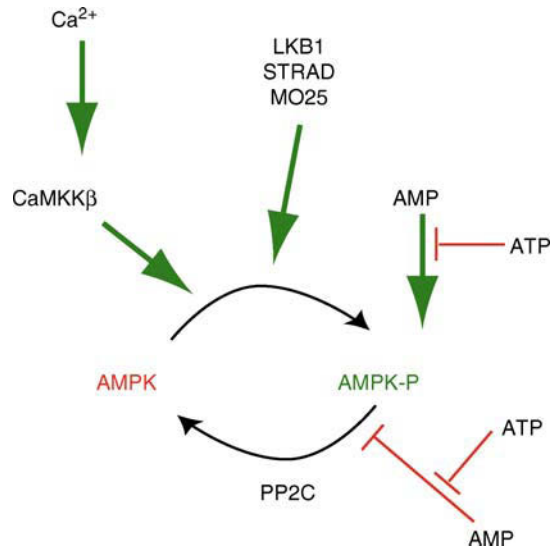
and cardiac muscle but probably also occurs elsewhere. The $\gamma 3$ isoform appears to be almost restricted to skeletal muscle.

Upstream kinases can phosphorylate the AMPK α subunits at Thr-172, thus activating the kinase complex by >100-fold. The major upstream kinase in most mammalian cells is a complex between the tumor suppressor, LKB1, and two accessory subunits, STRAD and MO25. LKB1, which does not require to be phosphorylated for activity, is completely inactive unless STRAD is bound to it, while MO25, a protein containing helical repeats distantly related to armadillo proteins, appears to stabilize the LKB1:STRAD complex. STRAD is a “pseudokinase” that has a domain related to a protein kinase domain. This domain has no catalytic activity but still binds ATP, although this is not required for its ability to activate LKB1.

In humans, the LKB1 complex is now known to act as an upstream kinase for the whole subfamily of AMPK-related protein kinases, including twelve other kinases with functions distinct from AMPK, as well as the two catalytic subunit isoforms of AMPK ($\alpha 1$ and $\alpha 2$). The LKB1 complex appears to be constitutively active, thus constantly phosphorylating Thr-172 on $\alpha 1$ and $\alpha 2$. However, the phosphate is normally rapidly removed by dephosphorylation by protein phosphatases, with the physiological phosphatase probably being a form of protein phosphatase-2C. Binding of AMP to the γ subunit of the AMPK complex markedly inhibits dephosphorylation by protein phosphatase-2C, thus providing a sensitive switch mechanism that converts AMPK to the active phosphorylated form (Fig. 1). AMP binding also allosterically activates AMPK up to tenfold, so that the system will remain hyper-activated until AMP dissociates (the combined effect of phosphorylation and allosteric activation is at least 1000-fold). The second CBS motif (in the N-terminal Bateman domain) of all γ subunits contains a sequence that resembles the sequence of the target sites on downstream substrates for AMPK, except that it contains a nonphosphorylatable residue in place of serine or threonine. An attractive hypothesis is that this “pseudosubstrate” sequence binds to the substrate-binding groove on the α subunit in the absence of AMP. Some of the residues within this pseudosubstrate sequence also appear to be directly involved with AMP binding, suggesting an obvious mechanism whereby AMP binding relieves this pseudosubstrate interaction and causes allosteric activation.

Both effects of AMP (inhibition of dephosphorylation and activation) are antagonized by high concentrations of ATP, which compete with AMP for binding at the Bateman domains. Thus, the AMPK system can monitor changes in the cellular AMP:ATP ratio.

As well as the activation of AMPK by increases in cellular AMP:ATP, due to phosphorylation by LKB1,



AMP-activated Protein Kinase. Figure 1 Mechanism of activation of AMPK by AMP and Ca^{2+} . In resting cells, the catalytic α subunit of the AMPK complex is continually phosphorylated at Thr-172 by the LKB1:STRAD:MO25 complex, but the phosphate is at the same time rapidly removed by protein phosphatases (probably a form of protein phosphatase-2C, PP2C). Binding of AMP due to the AMPK γ subunit, due to elevation of AMP in response to metabolic stress, inhibits dephosphorylation and thus tends to switch the AMPK complex to the active, phosphorylated form, where it is further activated allosterically by AMP. High concentrations of ATP antagonize both effects of AMP, by competing with AMP for binding at the γ subunit. In some cell types that express CaMKK β , elevation of cytosolic Ca^{2+} due to an external stimulus can cause increased phosphorylation of AMPK. This is not dependent on an increase in AMP, although it may be further accentuated by it.

AMPK can also be activated by a Ca^{2+} -mediated pathway involving phosphorylation at Thr-172 by the Ca^{2+} /calmodulin-dependent protein kinase, CaMKK β . CaMKK α and CaMKK β were discovered as the upstream kinase for the calmodulin-dependent protein kinases-I and -IV; they both activate AMPK in a Ca^{2+} /calmodulin-dependent manner in cell-free assays, although CaMKK β appears to much more active against AMPK in intact cells. Expression of CaMKK α and CaMKK β primarily occurs in neural tissues, but CaMKK β is also expressed in some other cell types. Thus, the Ca^{2+} -mediated pathway for AMPK activation has now been shown to occur in response to depolarization in rat neuronal tissue, in response to thrombin (acting via a Gq-coupled receptor) in endothelial cells, and in response to activation of the T cell receptor in T cells.

AMPK is also regulated by a number of cytokines, including ►adipokines secreted from adipocytes that

are thought to play a key role in the regulation of whole body energy balance. Thus, it is activated by the adipokine ▶leptin in skeletal muscle, where it stimulates energy expenditure by activating fatty acid oxidation whereas, conversely, it is inhibited by leptin in the hypothalamus. The latter appears to be involved in the ability of leptin to inhibit food intake, because other agents that stimulate AMPK in the hypothalamus, such as the gut hormone ghrelin, cannabinoids, and hypoglycemia, all stimulate food intake in rodents. AMPK is also activated in muscle and liver by the ▶adipokine ▶adiponectin, with activation of AMPK by ▶adiponectin in the liver being required for its hypoglycemic effects, and by the proposed “▶myokine” interleukin-6. The molecular mechanism(s) by which these cytokines regulate AMPK remain unclear at present.

Once activated, the AMPK system switches on catabolic pathways that generate ATP (upper entries in Table 2), such as the uptake and oxidation of fatty

acids and glucose, while at the same time inhibiting ATP-consuming processes (lower entries in Table 2) such as biosynthetic pathways involved in energy storage, and in cell growth and proliferation. AMPK achieves this both by direct phosphorylation of metabolic enzymes, and by phosphorylation of transcription factors or coactivators that regulate gene expression.

Drugs

The first pharmacological agent shown to activate AMPK was 5-aminoimidazole-4-carboxamide (AICA) riboside, also known as ▶acadesine. This adenosine analogue is taken up into cells by adenosine transporters and phosphorylated by adenosine kinase to the monophosphorylated form, AICA ribotide or ZMP. ZMP accumulates inside cells to higher concentrations than the concentration of AICA riboside present in the medium, and it mimics both effects of AMP on AMPK system (allosteric activation and inhibition of

AMP-activated Protein Kinase. Table 2 Metabolic effects of AMPK activation. In cases marked with an *asterisk, there is evidence that AMPK mediates its effects by modulating the target named, although it is not yet clear whether the protein is directly phosphorylated by AMPK

Metabolic process	Effect	Immediate target	Immediate effects	Tissue
Glucose uptake	↑	AS160?	↑ GLUT4 translocation	Muscle
Glucose uptake	↑	*Myocyte enhancer factor-2	↑ GLUT4 expression	Muscle
Glucose uptake	↑	?	↑ GLUT1 activity	Many cells
Glycolysis	↑	6-phosphofructo-2-kinase (cardiac isoform)	↑ Activity ↑ Fructose-2,6-bisphosphate	Cardiac myocytes
Glycolysis	↑	6-phosphofructo-2-kinase (inducible isoform)	↑ Activity ↑ Fructose-2,6-bisphosphate	Monocytes, macrophages
Fatty acid oxidation	↑	Acetyl-CoA carboxylase-2	↓ Activity, ↓ malonyl-CoA ↑ CPT1 activity	Liver, muscle, others
Mitochondrial biogenesis	↑	*PGC-1α?	↑ PGC-1α expression	Muscle, others?
Fatty acid synthesis	↓	Acetyl-CoA carboxylase-1	↓ Activity	All cells?
Fatty acid synthesis	↓	*SREBP-1c, *HNF-4α	↓ Expression ACC1, fatty acid synthase	Liver
Cholesterol synthesis	↓	HMG-CoA reductase	↓ Activity	Liver
Glycogen synthesis	↓	Muscle glycogen synthase	↓ Activity	Muscle
Gluconeogenesis	↓	TORC2, others?	↓ Expression PEP carboxykinase, glucose-6-phosphatase	Liver
Protein synthesis	↓	*Elongation factor-2 kinase	↓ Translation elongation	All cells?
Protein synthesis	↓	TSC2	↓ TOR, ↓ S6K1 ↓ Translation initiation	All cells?
Autophagy	↑	TSC2?	↓ TOR	All cells?
Glucose uptake	↓	?	↓ Insulin stimulation	Adipocytes
Lipolysis	↓	Hormone sensitive lipase (HSL)	↓ Adrenergic stimulation of lipolysis	Adipocytes

dephosphorylation), although it is less potent than AMP itself. Thus, AICA riboside can be used to activate AMPK in intact cells and in vivo. It has been used in animal models of obesity and insulin resistance such as the ob/ob mouse, the fa/fa rat and the fat-fed rat, where it improves glucose tolerance, increases insulin sensitivity and lowers blood pressure. Acadesine has also undergone clinical trials in humans, particularly as an acute treatment during coronary artery bypass graft surgery. These studies were initiated before it was realized that the drug was a potential AMPK activator, and the rationale was that it could be used to replenish adenine nucleotides lost from the cardiac muscle during periods of ischemia. These trials appear to have yielded inconclusive results regarding the efficacy of acadesine in such circumstances.

In 2001 it was reported that AMPK was activated by the ►**biguanide metformin**, and the following year it was reported that it was activated by the ►**thiazolidinediones**, ►**rosiglitazone**, and ►**pioglitazone**. ►**Metformin** is currently the most widely prescribed drug used to treat type 2 diabetes (current estimates are that it is used to treat 120 million worldwide), while the ►**thiazolidinediones** are another major drug class used to treat insulin resistance and type 2 diabetes. ►**Metformin** (and its sister drug ►**phenformin**) have no known target other than AMPK, and recent evidence involving mice in which the LKB1 gene was knocked down in liver (thus preventing activation of AMPK by the ►**biguanides**) supports the idea that the primary target of the drug is liver AMPK. Activation of liver AMPK decreases hepatic glucose production by downregulating expression of enzymes of gluconeogenesis, e.g. phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. ►**Metformin** and ►**phenformin** do not activate AMPK directly in cell-free assays but, surprisingly, appear to work indirectly by inhibiting complex I of the respiratory chain, thus increasing the cellular AMP:ATP ratio. This mechanism of action explains the main side effect of ►**phenformin**, i.e. lactic acidosis, which led to the withdrawal of the drug. There appears to be a much lower risk of lactic acidosis in the case of ►**metformin**, where gastrointestinal disturbance is the major side effect. However, there is evidence from animal studies that the major source of lactic acid produced in response to ►**biguanides** is the gut, and it is possible that inhibition of the respiratory chain in intestinal epithelial cells may explain the gastrointestinal side effects commonly reported during metformin use.

The thiazolidinediones have also been reported to act as inhibitors of the respiratory chain at high concentrations, and this appears to account for their ability to activate AMPK in cultured cells. However, the primary target of the thiazolidinediones appears to be the peroxisome proliferator-activated receptor- γ (►**PPAR- γ**), a member of the nuclear receptor superfamily expressed in adipocytes. One of the major effects of stimulation of ►**PPAR- γ** in adipocytes is the release of the

►**adipokine adiponectin**, and studies in ►**adiponectin-deficient mice** suggest that this accounts for many, although not all, of the effects of ►**thiazolidinediones**. AMPK is in any case a major target for ►**adiponectin** action, with knockdown of liver AMPK by expression of a dominant negative mutant preventing the hypoglycemic effects of ►**adiponectin**.

►**Diabetes Mellitus**

►**Antidiabetic Drug Other than Insulin**

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AMPA Receptors

The ionotropic glutamate receptors have been classified based upon their pharmacology and form three distinct subgroups. These are the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, N-methyl-D-aspartate (NMDA) receptors, and kainate (KA) receptors. AMPA receptors are responsible for the majority of fast excitatory synaptic transmission, and their over-activation is potentially excitotoxic. AMPA receptors conduct Na^+ , K^+ and sometimes Ca^{2+} in response to ligand binding. The specific conducting and kinetic properties depend on the receptor subunit composition as each mature AMPA receptor is assembled from four individual subunits. The influx of ions causes a fast excitatory postsynaptic response, supports NMDA receptor activation, and the Ca^{2+} component can also activate second messenger pathways, including many protein kinases.

►**Neurotransmitter Transporters**

►**Ionotropic Glutamate Receptors**

Amphetamine

Amphetamine and related compounds are indirect acting sympathomimetic agents that are frequently abused due to their stimulant properties on the central nervous system. Amphetamines act by inducing the

biogenic amine transporters to reverse or efflux neurotransmitter into the synapse. This drug-induced nonvesicular release of dopamine, norepinephrine and serotonin is thought to be the major action associated with the amphetamines. Clinically, the amphetamines are effective in the treatment of narcolepsy and Attention Deficit Hyperactivity Disorder (ADHD).

- Psychostimulants
- Neurotensin/Neuromedin N

Amphipathic

Possessing both hydrophilic and hydrophobic properties.

- Bile Acids

AMPK

- AMP-activated Protein Kinase

Amyloid

This is an extracellular deposition of an “insoluble” protein, which has adopted a β -sheet structure due to an unknown event that induced misfolding of unstable proteins. The name “amyloid” has been given according to the amyloid staining properties, which are similar to carbohydrate deposits, e.g., amyloid can be identified with congo red and seen under polarized light (birefringence test).

- Alzheimer’s Disease

A β Amyloid

A β amyloid is a 4 kD peptide which is the principle constituent of the Alzheimer amyloid found extracellularly in the brains of Alzheimer patients. A β amyloid is cleaved from a larger precursor protein, amyloid

precursor protein (APP). APP is a member of a family that includes various proteins. It is present in the dendrites, cell bodies and axons of neurons. Neuronal APP is probably the source of most of the A β amyloid deposited in the central nervous system of Alzheimer patients. APP is cleaved by various proteases, α -, β - and γ -secretase. The endopeptidase α -secretase cleaves within the A β region of APP, resulting in the secretion of the extracellular domain of APP; hence, the cleavage does not produce the A β peptide. In contrast, the β -secretase and γ -secretase cleavages do result in production of the A β peptide.

- Alzheimer’s Disease

Amyloid Precursor Protein

Amyloid precursor protein (APP) is the precursor of β -amyloid, the main component of senile plaques found in the brain of Alzheimer patients. The production of β -amyloid from APP to the cells from abnormal proteolytic cleavage of the amyloid precursor protein. Enzymes involved in this cleavage may be suitable targets for the therapy of Alzheimer’s disease.

- Alzheimer’s Disease

Amyotrophic Lateral Sclerosis (ALS)

ALS is a disorder of the motor neurons and the cortical neurons that provide their input. The disorder is characterized by rapidly progressive weakness and muscle atrophy. Most affected patients die of respiratory compromise and pneumonia after 2 to 3 years. There is prominent loss of motor neurons in the spinal cord and brainstem although the oculomotor neurons are spared. Large pyramidal motor neurons in layer V of motor cortex, which are the origin of the descending corticospinal tracts, are also lost.

It has been suggested that excitotoxic neurotransmitters such as glutamate participate in the death of motor neurons in ALS. This may be a consequence of diminished uptake of synaptic glutamate by an astroglial glutamate transporter, GLT1 (EAAT2) because overexpression of GLT1 in animals can delay the onset and the natural course of the disease.

ALS can run in families (10% of the cases) and superoxide dismutase 1 (SOD1) is an enzyme that has been found mutated in affected families; the majority of ALS patients, however, have normal SOD1. The importance of the SOD1 mutation is that transgenic mice expressing mutant human SOD1 develop a progressive degeneration of motor neurons that closely mimic the human disease. Disease causing mutations of SOD1, however, do not reduce the capacity of the enzyme to perform the catabolism of superoxide radicals.

► Neurotransmitter Transporters

► Neurotrophic Factors

Anabolic Steroids

Anabolic steroids increase muscle mass and strength. They are used by some athletes to enhance performance.

► Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

Anaesthetics

► General Anaesthetics

► Local Anaesthetics

Analeptics

The term analeptics refers to convulsants and respiratory stimulants (i.e. central nervous system stimulants). They comprise a reverse group of agents (for example amphifinazole and doxapram (respiratory stimulants) and strychnine, biculline and picrotoxin). Analeptics are mainly experimental drugs. Only amphifinazole and doxapram are occasionally used for the treatment of acute ventilatory failure.

Analgesia

► Analgesics

Analgesics

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Synonyms

Pain medication

Definition

Analgesics interfere with the generation and/or transmission of impulses following noxious stimulation (► **nociception**) in the nervous system. This can occur at peripheral and/or central levels of the ► **neuraxis**. The therapeutic aim is to diminish the perception of ► **pain**.

Mechanisms of Action

Analgesics aim at modulating the generation of noxious chemicals (e.g., prostaglandins) or the activation of neuronal receptors/ion channels transducing/transmitting noxious stimuli (e.g., peptide-, kinin-, monoamine-receptors, Na⁺ channels) [5]. Clinically used drugs include opioids, nonsteroidal antiinflammatory drugs (NSAIDs), serotonergic compounds, antiepileptics, and antidepressants (Table 1) [1]. Adrenergic agonists, excitatory amino acid (e.g., *N*-methyl-D-aspartate; NMDA) receptor antagonists, neurotrophin antagonists, peptide antagonists, kinin receptor antagonists, cannabinoids, and ion channel (e.g., ► **TRP**, ► **P2X**) blockers are currently under investigation but are not used routinely yet. ► **Local anaesthetics** are used for local and regional anesthetic techniques. Mixed drugs (e.g., tramadol) combine various mechanisms [3].

Opioids

Opioids act on heptahelical ► **G-protein-coupled receptors**. Three types of opioid receptors (μ , δ , κ) have been cloned. Additional subtypes (e.g., μ_1 , μ_2 , δ_1 , δ_2), possibly resulting from gene polymorphisms, splice variants or alternative processing have been proposed. Opioid receptors are localized and can be activated

Analgesics. Table 1 Analgesics

Drugs	Targets	Mechanisms	Functional consequences	Side effects
Opioids	G-protein coupled μ -, δ -, κ -receptors	$\downarrow$ cAMP $\downarrow$ Ca^{2+} currents $\uparrow$ K^+ currents	$\downarrow$ Excitability of peripheral and central neurons $\downarrow$ Release of excitatory neurotransmitters	μ , δ : sedation, nausea, euphoria/reward, respiratory depression, constipation κ : dysphoria/aversion, diuresis, sedation
NSAIDs	Cyclooxygenases (COX-1, COX-2)	$\downarrow$ Prostaglandins $\downarrow$ Thromboxanes	$\downarrow$ Sensitization of sensory neurons $\uparrow$ Inhibition of spinal neurons	Nonselective: gastrointestinal ulcers, perforation, bleeding, renal impairment COX-2: thrombosis, myocardial infarction, stroke
Serotonin agonists	G-protein coupled 5-HT receptors 5-HT ₃ : ion channels	$\downarrow$ cAMP (5-HT ₁) $\uparrow$ cAMP (5-HT ₄₋₇) $\uparrow$ PLC (5-HT ₂)	$\downarrow$ Release of excitatory neuropeptides $\downarrow$ Neurogenic inflammation $\uparrow$ vasoconstriction	Myocardial infarction, stroke, peripheral vascular occlusion
Antiepileptics	Na^+ , Ca^{2+} channels GABA receptors	$\downarrow$ Na^+ currents $\downarrow$ Ca^{2+} currents $\uparrow$ GABA receptor activity	$\downarrow$ Excitability of peripheral and central neurons $\downarrow$ Release of excitatory neurotransmitters	Sedation, dizziness, cognitive impairment, ataxia, hepatotoxicity, thrombocytopenia
Antidepressants	Noradrenaline/5-HT transporters Na^+ , K^+ channels	$\downarrow$ Noradrenaline/5-HT reuptake $\downarrow$ Na^+ currents $\uparrow$ K^+ currents	$\downarrow$ Excitability of peripheral and central neurons	Cardiac arrhythmia, myocardial infarction, sedation, nausea, dry mouth, constipation, dizziness, sleep disturbance, blurred vision

along all levels of the neuraxis including peripheral and central processes of primary sensory neurons (► **nociceptors**), spinal cord (interneurons, projection neurons), brainstem, midbrain, and cortex. All opioid receptors couple to G-proteins (mainly G_i/G_o) and subsequently inhibit adenylyl cyclase, decrease the conductance of voltage-gated ► **Ca^{2+} channels** and/or open rectifying ► **potassium channels**. These effects ultimately result in decreased neuronal activity. The prevention of Ca^{2+} influx inhibits the release of excitatory (pronociceptive) neurotransmitters. A prominent example is the suppression of ► **tachykinin** (substance P) release from primary sensory neurons both within the spinal cord and from their peripheral terminals within injured tissue. At the postsynaptic membrane, opioids produce hyperpolarization by opening K^+ channels, thereby preventing excitation or propagation of action potentials in second order projection neurons. In addition, opioids inhibit sensory neuron-specific tetrodotoxin-resistant ► **Na^+ -channels**, ► **TRPV1 channels** and excitatory postsynaptic currents evoked by ► **glutamate receptors** (e.g., NMDA) in the spinal cord. The result is decreased transmission of nociceptive stimuli at all levels of the neuraxis and profoundly reduced perception of pain

[2, 3]. Endogenous opioid receptor ligands are derived from the precursors proopiomelanocortin (encoding β -endorphin), proenkephalin (encoding Met-enkephalin and Leu-enkephalin) and prodynorphin (encoding dynorphins). These peptides contain the common Tyr-Gly-Gly-Phe-[Met/Leu] sequence at their amino terminals, known as the opioid motif. β -Endorphin and the enkephalins are potent antinociceptive agents acting at μ and δ receptors. Dynorphins can elicit both pro- and antinociceptive effects via κ -opioid and/or NMDA receptors. A fourth group of tetrapeptides (endomorphins) with yet unknown precursors do not contain the pan-opioid motif but bind to μ -receptors with high selectivity. Opioid peptides and receptors are expressed throughout the central and peripheral nervous system, in neuroendocrine tissues and in immune cells [3, 4].

Nonsteroidal Antiinflammatory Drugs

NSAIDs inhibit ► **cyclooxygenases** (COX), the enzymes that catalyze the transformation of arachidonic acid (a ubiquitous cell component generated from phospholipids) to prostaglandins and thromboxanes. Two isoforms, COX-1 and COX-2, are constitutively expressed in peripheral tissues and in the central nervous

system. In response to injury and inflammatory mediators, (e.g., ►**cytokines**, growth factors) both isoforms can be upregulated, resulting in increased concentrations of prostaglandins. In the periphery, prostaglandins (mainly PGE₂) sensitize nociceptors by phosphorylation of ion channels (e.g., Na⁺, TRPV1) via ►**EP receptor** activation. As a result, nociceptors become more responsive to noxious mechanical (e.g., pressure, hollow organ distension), chemical (e.g., acidosis, ►**bradykinin**, ►**neurotrophic factors**) or thermal stimuli. In the spinal cord PGE₂ blocks glycinergic neuronal inhibition, enhances excitatory amino acid release, and depolarizes ascending neurons. These mechanisms facilitate the generation of impulses within nociceptors and their transmission through the spinal cord to higher brain areas [5]. By blocking one (selective COX-2 inhibitors) or both enzymes (nonselective NSAIDs) prostaglandin formation diminishes. Subsequently nociceptors become less responsive to noxious stimuli and spinal neurotransmission is attenuated [3].

Serotonergic Drugs

►**Serotonin** (5-hydroxytryptamine; 5-HT) is a monoamine neurotransmitter found in the sympathetic nervous system, in the gastrointestinal tract, and in platelets. It acts on 5-HT receptors expressed at all levels of the neuraxis and on blood vessels. Within the dorsal horn of the spinal cord serotonergic neurons contribute to endogenous pain inhibition. With the exception of 5-HT₃ (a ligand-gated ion channel), 5-HT receptors are G-protein coupled receptors. 5-HT_{1B/1D} agonists (triptans) have been studied extensively and are effective against neurovascular (migraine, cluster) headaches. Migraine is thought to be related to the release of neuropeptides (e.g., ►**calcitonin gene related peptide**) from trigeminal sensory neurons innervating meningeal and intracranial blood vessels. This leads to vasodilation, an inflammatory reaction, and subsequent pain. Triptans inhibit neurogenic inflammation via 5-HT_{1D} receptors on trigeminal afferents, with possible additional sites of action on thalamic neurons and in the periaqueductal grey. The activation of vascular 5-HT_{1B} receptors constricts meningeal (and coronary) vessels. The latter effects have stimulated a search for nonvasoconstrictor approaches such as highly selective 5HT_{1D} and 5HT_{1F} agonists. However, none of them demonstrated clinical antimigraine effects so far [3].

Antiepileptic Drugs

Antiepileptics are used in ►**neuropathic pain** resulting from lesions to the peripheral (e.g., diabetes, herpes) or central nervous system (e.g., stroke). Such syndromes have been attributed to ectopic activity in sensitized nociceptors from regenerating nerve sprouts, recruitment of previously “silent” nociceptors, and/or spontaneous neuronal activity. This may result in sensitization

of primary afferents and subsequent sensitization of second- and third order ascending neurons. Among the best studied mechanisms are the increased expression and trafficking of ion channels (e.g., Na⁺, Ca²⁺, TRP) and increased activity at ►**glutamate (NMDA) receptor** sites. The mechanisms of action of antiepileptics include neuronal membrane stabilization by blockage of pathologically active voltage-sensitive Na⁺ channels (carbamazepine, phenytoin, lamotrigine, topiramate), blockage of voltage-dependent Ca²⁺ channels (gabapentin, pregabalin), inhibition of presynaptic release of excitatory neurotransmitters (gabapentin, lamotrigine) and enhancing the activity of γ-aminobutyric acid ►**(GABA) receptors** (topiramate) [3].

Antidepressants

Antidepressants are used in the treatment of neuropathic pain and headache. They include the classic tricyclic compounds and are divided into nonselective noradrenaline/5-HT reuptake inhibitors (e.g., amitriptyline, imipramine, clomipramine, venlafaxine), preferential noradrenaline reuptake inhibitors (e.g., desipramine, nortriptyline) and selective 5-HT reuptake inhibitors (e.g., citalopram, paroxetine, fluoxetine). The reuptake block leads to a stimulation of endogenous monoaminergic pain inhibition in the spinal cord and brain. In addition, tricyclics have NMDA receptor antagonist, endogenous opioid enhancing, Na⁺ channel blocking, and K⁺ channel opening effects which can suppress peripheral and central sensitization. Block of cardiac ion channels by tricyclics can lead to life-threatening arrhythmias. The selective 5-HT transporter inhibitors have a different side effect profile and are safer in cases of overdose [3].

Clinical Use and Side Effects

Analgesics are used in both acute and chronic pain. Whereas acute (e.g., postoperative, posttraumatic) pain is generally amenable to drug therapy, chronic pain is a complex disease in its own right and needs to be differentiated into malignant (cancer-related) and nonmalignant (e.g., musculoskeletal, ►**neuropathic**, inflammatory) pain. Acute and cancer-related pain is commonly treatable with opioids, NSAIDs, and/or local anesthetic blocks. Chronic nonmalignant pain requires a multidisciplinary approach encompassing various pharmacological and nonpharmacological (e.g., psychological, physiotherapeutic) treatment strategies. Various routes of drug administration (e.g., oral, intravenous, subcutaneous, intrathecal, ►**epidural**, topical, intraarticular, transnasal) are used, depending on the clinical circumstances. Local anesthetics are used topically and in regional (e.g., epidural) anesthetic techniques for the treatment of acute (e.g., associated with surgery, child birth) and some selected chronic pain syndromes.

Opioids

Opioids are the most effective drugs for severe acute and cancer-related chronic pain. They do not improve quality of life in chronic noncancer pain. The commonly available agents (e.g., morphine, codeine, methadone, fentanyl and its derivatives) are μ -agonists. Naloxone is a nonselective antagonist at all three receptors. Partial agonists must occupy a greater fraction of the available pool of functional receptors than full agonists to induce a response (e.g., analgesia) of equivalent magnitude. Mixed agonist/antagonists (e.g., buprenorphine, butorphanol, nalbuphine, pentazocine) may act as agonists at low doses and as antagonists (at the same or a different receptor) at higher doses. Such compounds typically exhibit ceiling effects for analgesia and they may elicit an acute **▶withdrawal** syndrome when administered together with a pure agonist. All three receptors (μ , δ , κ) mediate analgesia but differing side effects. μ -Receptors mediate respiratory depression, sedation, reward/euphoria, nausea, urinary retention, biliary spasm, and constipation. κ -Receptors mediate dysphoric, aversive, sedative, and diuretic effects, but do not mediate constipation. δ -Receptors mediate reward/euphoria and, to a lesser degree, respiratory depression and constipation. **▶Tolerance** and physical **▶dependence** occur with prolonged administration of pure agonists, and abrupt discontinuation or antagonist administration can result in a withdrawal syndrome [3]. Opioids are effective in the periphery (e.g., topical or intraarticular administration, particularly in inflamed tissue), at the spinal cord (intrathecal or epidural administration), and systemically (e.g., intravenous or oral administration). The clinical choice of a particular compound is based on pharmacokinetic considerations (route of administration, desired onset or duration, lipophilicity) and on side effects associated with the respective route of drug delivery. Dosages are dependent on patient characteristics, type of pain and route of administration. Systemically and spinally administered μ -opioids can produce similar side effects, depending on dosage and rostral/systemic redistribution. For intrathecal application lipophilic drugs are preferred because they are trapped in the spinal cord and less likely to migrate to the brain within the cerebrospinal fluid. Adverse side effects can be minimized by careful dose titration and close patient monitoring, or can be treated by comedication (e.g., laxatives) or naloxone. The peripheral application of small, systemically inactive doses is devoid of side effects. Current research aims at the development of opioids with restricted access to the brain [2, 4].

Nonsteroidal Antiinflammatory Drugs

Less severe pain states (e.g., arthritis, menstruation, headache, minor surgery) are commonly treated with nonselective NSAIDs (e.g., aspirin, ibuprofen, indomethacin, diclofenac). NSAIDs are mostly used orally.

Some agents are available for parenteral, rectal, or topical application. Over-the-counter availability and self medication have led to frequent abuse and toxicity. Side effects have been attributed to COX-1 induced blockade of thromboxane production and impairment of platelet function (gastrointestinal and other bleeding disorders), decrease of tissue-protective prostaglandins (gastrointestinal ulcers, perforation), and decrease of renal vasodilatory prostaglandins (nephrotoxicity). The development of selective COX-2 inhibitors was driven by the assumption that COX-2 expression is selectively induced in inflamed tissue and that the constitutive tissue-protective COX-1 would be spared. It has now become clear that COX-2 expression is constitutive in many tissues (e.g., gastrointestinal epithelium, vascular endothelium, spinal cord) and COX-2 inhibition may exacerbate inflammation, impair ulcer healing, and decrease formation of vasoprotective prostacyclin. Selective COX-2 inhibitors confer an increased risk of thrombosis, myocardial infarction, hypertension, and stroke. Both classes of COX inhibitors can cause rare anaphylactic reactions. Acetaminophen (paracetamol) has relatively weak anti-inflammatory and antiplatelet activity. It is used for osteoarthritis, headache, and fever [1].

Serotonergic Drugs

Triptans can be applied orally, subcutaneously, or transnasally and have been used in the treatment of migraine. All triptans narrow coronary arteries via 5-HT_{1B} receptors by up to 20% at clinical doses and should not be administered to patients with risk factors or manifest coronary, cerebrovascular, or peripheral vascular disease. Some triptans have the potential for significant drug–drug interactions (e.g., with monoamine oxidase inhibitors, propranolol, cimetidine, hepatic P450-metabolized medications, P-glycoprotein pump inhibitors). Rational use of triptans should be restricted to patients with disability associated with migraine [1].

Antiepileptic Drugs

Antiepileptics have been used for neuropathic pain and for migraine prophylaxis. They are frequently coadministered with antidepressants. The most common adverse effects are impaired mental (somnolence, dizziness, cognitive impairment, fatigue) and motor function (ataxia) which limits clinical use, particularly in elderly patients. Serious side effects have been reported, including hepatotoxicity, thrombocytopenia and life-threatening dermatologic and hematologic reactions. Plasma drug concentrations should be monitored [1].

Antidepressants

Antidepressants are used in neuropathic pain and migraine prophylaxis. Tricyclics require monitoring of plasma drug concentrations to achieve optimal effect

and avoid toxicity, unless sufficient pain relief is obtained with low doses (up to 75 mg/day of imipramine or amitriptyline). In patients with ischemic heart disease there may be increased mortality from sudden arrhythmia, and in patients with recent myocardial infarction, arrhythmia, or cardiac decompensation tricyclics should not be used at all. Tricyclics also block histamine, cholinergic and adrenergic receptor sites. Adverse events include sedation, nausea, dry mouth, constipation, dizziness, sleep disturbance, and blurred vision [1].

- Non-steroidal Anti-inflammatory Drugs
- Opioid Systems
- Local Anaesthetics
- Voltage-dependent Na⁺ Channels

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Analogous Proteins

Two proteins with related folds but unrelated sequences are called analogous. During evolution, analogous proteins independently developed the same fold.

- Bioinformatics

Anandamide

A natural synthesised cannabinoid interacting with the cannabinoid receptor I and II. In addition, anandamide blocks receptor-independent all LVA-calcium channels.

- Voltage-dependent Ca²⁺ Channels
- Endocannabinoids

Anaphase Promoting Complex (APC)

The APC is a complex, which is activated during mitosis and initiates anaphase by targeting key cell cycle regulators for proteasomal degradation. As proteolysis is irreversible cell cycle progression cannot be reverted. Targets are mitotic cyclins and securin. Proteolysis of securin activates separase, which cleaves cohesins thereby allowing detachment of sister chromatids.

- Ubiquitin/Proteasome

Anaphylactic Shock

The term anaphylactic shock describes a severe generalized type I allergic reaction associated with cardiovascular shock, airway constriction and heart arrhythmias, which, if left untreated, may cause death.

- Allergy

Anchoring Protein

- Adaptor Proteins
- A Kinase Anchoring Proteins (AKAPs)

Andersen's Syndrome

Andersen's syndrome is a rare disorder characterized by periodic paralysis, cardiac arrhythmias, and dysmorphic features.

- K⁺-Channels

Androgen

Androgens, represented by testosterone, are male sex hormones involved in reproduction, behavior, and bone and muscle growth.

- Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
- Selective Sex Steroid Receptor Modulators

Androgen Receptor

- Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

Anemia, Macrocytic Hyperchromic

Macrocytic or magaloblastic anemia is caused by disturbances of DNA synthesis. It occurs, for example, in both folic acid and vitamin B12 deficiencies. Hematopoiesis is slowed down due to reduced DNA synthesis and a reduced number of abnormally large (macrocytic) and hemaglobin-rich (hyperchromic) erythrocytes is released.

- Vitamin B12

Angel Dust

- Psychotomimetic Drugs

Angina Pectoris

A reversible attack of chest discomfort, usually caused by an imbalance between the oxygen demand of the working heart muscle and the insufficient supply through narrow, atherosclerotic coronary arteries.

- Atherosclerosis

Angiogenesis and Vascular Morphogenesis

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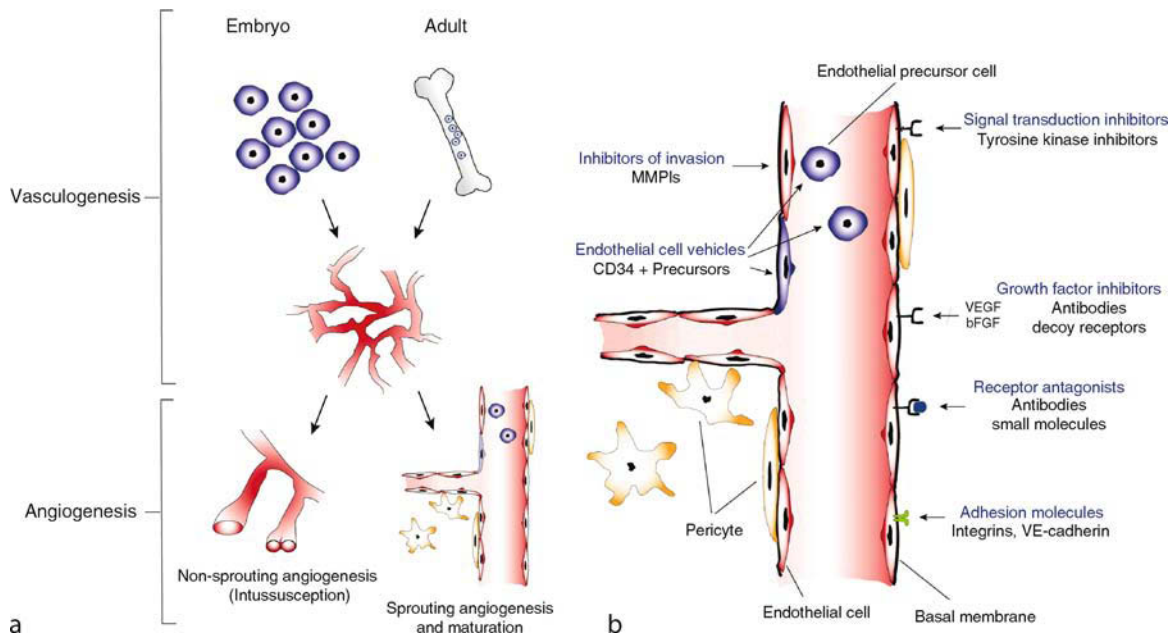
Definition

Angiogenesis and vascular morphogenesis comprise all mechanisms and processes that lead to the development of new blood vessels. These include vasculogenesis, sprouting and non-sprouting angiogenesis, vessel assembly and maturation, and vascular remodeling. The formation of new blood and lymphatic vessels takes place primarily during embryonic development and blood neo-vessel formation is physiologically limited to few organs in the healthy adult such as the female reproductive system. Pathologic angiogenesis is widespread and is associated with diseases as diverse as tumors, wound healing, inflammatory diseases, skin diseases, eye diseases, and joint diseases.

Basic Mechanisms

Vasculogenesis and Angiogenesis

Blood vessels arise during embryogenesis by vasculogenesis and angiogenesis (Fig. 1a). These two processes are tightly regulated and display common stimulatory and inhibitory pathways [1]. During vasculogenesis, endothelial cells differentiate from mesodermal origin while during angiogenesis endothelial cells are formed from preexistent ones. Establishment of the vascular system involves formation of the vitelline (yolk sac) vasculature, the allantois (precursor of the umbilical vasculature of the placenta) and the cardiovascular system that ultimately merge to form a continuous vascular system. Differentiation of vascular lineages is synchronized and organized in parallel to other embryonic lineages. Contribution from the other germ layers (ectoderm and endoderm) to vasculogenesis has not been reported even though recent evidence has demonstrated the contribution of ectodermal tissue as a source of vascular differentiating factors as well as a support to form vascular tubes. For instance, the contribution of the peripheral neural system to vascular development has recently been demonstrated. Nerve-derived ►vascular endothelial growth factor (VEGF) is necessary for arteriogenesis from a primitive capillary plexus. In a similar but inverse manner, the endothelium also appears to exert an active organ differentiation-inducing function, e.g., endothelial precursor cells have



Angiogenesis and Vascular Morphogenesis. Figure 1 Molecular mechanisms of vasculogenesis and angiogenesis (a) and the corresponding targets for therapeutic intervention. The primary formation of blood vessels occurs through mechanisms of vasculogenesis. Vasculogenesis refers to the formation of a vascular network from precursor cells (angioblasts) as it occurs developmentally by *in situ* differentiation or in the adult by distal recruitment of angioblastic stem cells from the bone marrow. The secondary level of vascular morphogenesis describes the angiogenic formation of blood vessels. Angiogenesis refers to the formation of vessels and vascular networks from preexisting vascular structures. This can occur through classical sprouting angiogenesis or through mechanisms of non-sprouting angiogenesis. The growing vascular network assembles and matures, eventually allowing directional blood flow. Pharmacological intervention of angiogenesis is based on different strategies interfering directly or indirectly with the endothelium as indicated in (b).

been shown to have an inducing effect on organ development in the pancreas and the liver.

The first stages of blood vessel formation involve the differentiation of mesodermal precursor cells into a plexus of angioblasts followed by their organization into angioblast cords taking the appearance of a hollow tube [2]. This first primitive vascular plexus expands by angiogenesis, the sprouting of new capillaries from preexisting vessels, and intussusception, a process during which interstitial tissue columns are inserted into the lumen of preexisting vessels (also termed non-sprouting angiogenesis; Fig. 1a). Sprouting and non-sprouting angiogenesis contribute to an increasing complexity of the growing vascular network. The network assembles and matures by the recruitment of pericytes and smooth muscle cells, eventually allowing the directional flow of blood. The morphogenic events leading to a mature vascular network involve several additional steps including vessel assembly, maturation, and acquisition of vessel identity and organotypic differentiation.

Vessel identity represents one of the major differentiation processes during blood vessel formation. Arteries and veins are structurally and functionally

distinct. It was long assumed that arteries and veins differentiate in response to differing blood flow rates and pressure gradients. Yet, the recent identification of molecules with an arteriovenous asymmetric expression pattern (arterial markers: **e**phrinB2, Notch 1, Delta-like 4 [Dll4], Connexin-40, Neuropilin-1, Bmx; venous markers: EphB4, Neuropilin-2) has paved the way for the genetic understanding of arteriovenous differentiation. Emerging is a concept of distinct transcriptional programs driving the differentiation of different endothelial lineages (arterial differentiation: sonic hedgehog → VEGF → VEGFR → FoxC1/FoxC2 → Notch → Notch downstream transcription factors [Hey1/Hey2 {mammals}; Gridlock {zebrafish}]; venous differentiation: transcription factor COUP-TFII [maintains venous differentiation by inhibiting Notch pathway; lymphatic differentiation: transcription factor Prox1]).

Molecular Regulators of the Angiogenic Cascade

The supply of oxygen and nutrients is a critical determinant for mammalian cell survival. Therefore, cells are located within a distance of 100 μm to maximally 150 μm of blood vessels. Multicellular

organisms growing beyond this size must recruit new blood vessels in order to achieve growth by mechanisms of vasculogenesis and angiogenesis. The regulation of this process is tightly controlled by a balance of pro- and antiangiogenic molecules. To date, more than

20 stimulators and 20 inhibitors have been identified (Table 1) and the composition of the “angiogenic cocktail” has not been well defined for most situations involving angiogenesis. Of the many angioregulatory molecules, well-defined families of molecules, i.e., the

Angiogenesis and Vascular Morphogenesis. Table 1 Positive and negative endogenous regulators of angiogenesis (noncomprehensive list)

Stimulators	Inhibitors
Peptide growth factors	Proteolytic peptides
VEGF-A, -B, -C, -D, -E	Angiostatin (plasminogen fragment)
PIGF	Vasostatin (calreticulin fragment)
Ang-1, Ang-2, ANGPTL4	α 6 IV NC1 domain (collagen α 1 IV fragment)
FGF-1, -2	Endostatin (collagen α 1 XVIII fragment)
PDGF-BB	Tumstatin (collagen α 3 IV fragment)
EGF	Canstatin (collagen α 2 IV fragment)
TGF- α , TGF- β	Arresten (collagen α 1 IV fragment)
HGF	Restin (α 1 Collagen IV NC1 fragment)
IGF-1, -2	Endorepellin (Perlecan fragment)
	Anastallin (fibronectin fragment)
	Antithrombin III fragment
Platelet-derived regulators	Inhibitors of enzymatic activity
PD-ECGF, TP	TIMP-1, -2, -3, -4
NPY	PAI-1, -2
Multifunctional cytokines/immune mediators	Multifunctional cytokines/immune mediators
TNF- α (low dose)	TNF- α (high dose)
Chemokines	Chemokines
MCP-1 (CCL2)	PF-4
IL-8 (CXCL8)	IP-10
Gro- α , - β , - γ , (CXCL1, 2, 3)	Gro- β
ENA-78 (CXCL5)	
NAP-2 (CXCL7)	
GCP-2 (CXCL2)	
Enzymes	Extracellular matrix molecules
Angiogenin (ribonuclease A homolog)	Thrombospondins
Hormones	Fibulin-5
Estrogens	Hormones/metabolites
Prostaglandin-E ₁ , -E ₂	2-ME
Follistatin	Proliferin-related protein
Hyaluronan oligosaccharides	
Gangliosides	Oligosaccharides
Hematopoietic growth factors	Hyaluronan, HMW species
Erythropoietin	
G-CSF	
GM-CSF	

Abbreviations: VEGF, vascular endothelial growth factor; -A, -B, -C, -D, -E; PIGF, placenta growth factor; Ang, angiopoietin, ANGPTL, angiopoietin like FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; TNF, tumor necrosis factor; MCP, monocyte chemoattractant protein; IL, interleukin; PD-ECGF, platelet-derived endothelial cell growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; TIMP, tissue metalloproteinase inhibitor; PAI, plasminogen activator inhibitor; PF, platelet factor; IP-10, interferon- γ -inducible protein-10; 2-ME, 2-Methoxyestradiol; HMW, high molecular weight, TP, Thymidine Phosphorylase, NPY, neuropeptide Y.

VEGFs, the ►angiopoietins, the Ephrins and Notch molecules stand out as they act specifically or preferentially on the vascular system and have, thus, to be considered as key regulatory molecules of the angiogenic cascade [3].

The best characterized angiogenic signaling pathway is VEGF. This has been unambiguously demonstrated through gene targeting experiments in mice which have shown that disruption of just one VEGF allele is not compatible with life and leads to early embryonic lethality. VEGF, now designated VEGF-A, is a member of a family of growth factors comprising VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E [4]. VEGF molecules exert their vascular specificity through the limited expression of their corresponding receptors, VEGFR-1, VEGFR-2, and VEGFR-3 which are differentially expressed by blood and lymphatic endothelial cells, respectively. Additionally, ►placenta growth factor (PlGF) is a VEGF related molecule (PlGF-1 and PlGF-2) which exclusively binds to VEGFR-1. PlGF is dispensable for embryonic and reproductive angiogenesis but plays important roles in pathological angiogenesis as it relates to tumor growth or cardiac ischemia. Despite its requisite role in angiogenesis, VEGF must act in concert with other growth factors. The angiopoietins (Ang-1 and Ang-2) have been identified as ligands for the endothelial cell receptor tyrosine kinase Tie-2. Gene ablation experiments in mice suggested a role of the Ang/Tie-2 system in vessel remodeling and stabilization of the growing neo-blood and -lymphatic vasculature. There are now four members of the angiopoietin (Ang) family, although Ang-3 and Ang-4 may represent widely diverged counterparts of the same gene locus in mouse and man. All of the known angiopoietins bind to Tie-2 and no ligand has yet been solidly established for the second Tie receptor, Tie-1.

More recently, the role of axon-guidance receptors and ligands during developmental angiogenesis has emerged as a focus in the field of angiogenesis research. These families of molecules involve Semaphorin/Neuropilin/Plexin, ephrin/Eph, Slit/robo, and Netrin/UNC. The ►Eph receptor tyrosine kinases comprise the largest known family of receptor tyrosine kinase receptors and interact in a specific, yet somewhat promiscuous manner with their corresponding ephrin ligands. Although initially characterized as regulators of axonal outgrowth in the nervous system, gene inactivation experiments in mice have revealed key roles for ephrinB2 and its EphB2, EphB3, and EphB4 receptors during vascular development. Mouse embryos lacking ephrinB2 or EphB4 (or the combination of EphB2 and EphB3) suffer dramatic defects in early angiogenic remodeling that are similar to those seen in mice lacking Ang-1 or Tie-2. Moreover, ephrinB2 and EphB4 display remarkable asymmetric

arterio-venous expression patterns, with ephrinB2 selectively marking arterial vessels and EphB4 preferentially being expressed by venous endothelial cells. More recently, several reports have associated class A Eph receptor signaling with tumor-associated angiogenesis. Another family of molecules, Notch receptors and their ligands Delta-like and Jagged, have been associated to venous and arterial specification during development and to also play critical roles during tumor angiogenesis. Dll4-deficient mice display the same vascular haploinsufficiency as VEGF with embryonic lethality of heterozygously targeted mice. Dll4 binds to Notch 1 and 4 receptors inducing a cascade of events leading to the up-regulation of the transcriptional repressors, Hey1 and Hey2. Dll4 expression is strongly up-regulated during angiogenesis. Its expression is induced by VEGF and it acts as a negative feedback regulator that restrains vascular sprouting and branching. Consistent with this role, the genetic deletion or manipulatory inhibition of Dll4 results in excessive, nonproductive angiogenesis and is therefore considered and explored as an attractive tumor angiogenesis target.

Angiogenic activation induces a specific gene expression program in endothelial cells enabling the cells to execute the complex molecular tasks required to grow new blood vessels. The invasive ingrowth of angiogenic endothelial cells involves a set of adhesion molecules including the integrin heterodimers $\alpha_v\beta_3$ and $\alpha_v\beta_5$ as well as the homotypic endothelial cell-specific adhesion molecule VE-cadherin. Likewise, sprouting endothelial cells display a shift of their proteolytic balance toward a proinvasive phenotype, involving the plasminogen activator (tPA and uPA) and plasminogen activator inhibitor system (PAI). Angiogenic endothelial cells deposit their own extracellular matrix, rearrange their cytoskeleton, and activate their proliferative machinery. All of these molecular systems are extensively being explored to therapeutically interfere with the angiogenic process.

Angiogenesis in Pathological Conditions

Abnormal vessel growth is involved in numerous pathological conditions. Pioneering work more than 35 years ago showed that the growth of solid tumors is critically dependent on the supply with new blood vessels. While some of this supply may be provided by mechanisms of vessel cooption, the process whereby a growing tumor is preying on the preexistent vasculature, the primary mechanism of tumor vascularization appears to be the angiogenic growth of blood vessels from the tumor neighboring blood vessels. Tumor cells release proangiogenic growth factors, such as VEGF, which diffuse into nearby tissues and bind and activate receptors on endothelial cells of preexisting blood vessels. Secretion of proteolytic enzymes, such as matrix metalloproteinases (MMPs) results in the

degradation of basement membrane and extracellular matrix components, allowing endothelial cells to invade and proliferate, and form new lumen containing vessels. Tumor angiogenesis involves the specific molecular regulators of the angiogenic cascade as they have been identified during developmental or reproductive angiogenesis. However, tumor angiogenesis also involves additional mechanisms that include the pleiotrophic angiogenic growth factors, for example as a consequence of the inflammatory response usually associated with tumor growth.

In addition to tumor angiogenesis, an increasing list of diseases is now recognized to critically depend on either increased or reduced angiogenesis. For example, increased angiogenesis occurs during diabetic retinopathy, macula degeneration, arthritic joint diseases, and hyperproliferative skin diseases. In turn, reduced angiogenesis may have a negative impact on wound healing and the regenerative processes associated with ischemic diseases as they occur in the heart or during peripheral limb ischemia.

Pharmacological Intervention

Both angio-inhibitory as well as angio-stimulatory therapies are presently being explored extensively for a number of indications [5]. Angio-inhibitory therapies are being developed most intensely to therapeutically interfere with tumor angiogenesis and tumor growth (Fig. 1b). Other major antiangiogenic therapies that are currently in clinical development target retinal diseases (diabetic retinopathy, macular degeneration), joint diseases (arthritis), as well as hyperproliferative skin diseases (psoriasis). In February 2004, the US Food and Drug Administration approved Bevacizumab, a humanized anti-VEGF-A monoclonal antibody for the treatment of metastatic colorectal cancer in combination with 5-fluorouracil-based chemotherapy. In December 2004, the FDA approved pegaptinib, an aptamer that blocks VEGF-A₁₆₅, for the treatment of the neovascular form of age-related macular degeneration. In turn, angio-stimulatory therapies are being developed to stimulate wound healing angiogenesis, peripheral limb ischemia, and arteriogenic growth of collateral vessels during cardiac ischemia.

Antiangiogenic Tumor Targeting

Antiangiogenic tumor targeting is conceptually a particularly attractive therapeutic avenue for a number of reasons: (i) As an oncofetal mechanism that is mostly downregulated in the healthy adult, targeting of angiogenesis should lead to minimal side effects even after prolonged treatment, (ii) tumor-associated angiogenesis is a physiological host mechanism and its pharmacological inhibition should, consequently, not lead to the development of resistance, (iii) each tumor

capillary potentially supplies hundreds of tumor cells and the targeting of the tumor vasculature should, thus, lead to a potentiation of the antitumorigenic effect, and (iv) in contrast to the interstitial location of tumor cells, direct contact of the vasculature to the circulation allows efficient access of therapeutic agents.

A number of approaches have been taken to inhibit tumor angiogenesis and other diseases involving angiogenesis (Table 2). Pharmacological inhibition of angiogenesis is aimed at interfering with the angiogenic cascade or the immature neovasculature. Pharmacological agents may be synthetic or semisynthetic substances, endogenous inhibitors of angiogenesis, or biological antagonists of the angiogenic cascade. In contrast, vascular targeting is aimed at utilizing specific molecular determinants of the neovasculature for the delivery of a biological, chemical, or physical activity that will then locally act angiocidal or tumoricidal. Moreover, in addition to their role on tumor cells, chemotherapeutic agents contribute may exert an antiangiogenic effect by targeting bone-marrow-derived proangiogenic cells, dividing endothelial cells from sprouting vessels, and endothelial cell progenitor incorporation to the lumen of growing vessels.

A comprehensive website summarizing the status of tumor antiangiogenic compounds in various stages of clinical trial is maintained by the National Cancer Institute at: ► www.cancer.gov/clinicaltrials/developments/anti-angio-table. Following is a survey of the most important substances currently in clinical development.

Specific Synthetic and Biological Antagonists of the Angiogenic Cascade

Specific inhibition of any of the key regulators of the angiogenic cascade is one of the most specific and selective ways to interfere with angiogenesis. A number of experimental strategies have been taken to interfere with the interaction of VEGF with its receptors. These include antisenses, ribozymes (i.e., Angiozyme in clinical trial Phase I) and antibody approaches to inhibit VEGF, the development of small molecular weight antagonists to the VEGF receptors, as well as the use of soluble VEGF receptors. A number of new generation small molecular weight VEGF receptor antagonists are rapidly proceeding in various stages of clinical trials (Table 3).

One of the most important steps to block angiogenesis has been made in targeting VEGF-A using the humanized antibody, Bevacizumab (Avastin) or a chimeric, soluble VEGF receptor ("VEGF-trap") which is also undergoing clinical development as an anti-cancer agent. Bevacizumab is a nonimmunogenic, 93% humanized murine monoclonal antibody that binds all VEGF-A isoforms. Bevacizumab has been clinically approved in 2004 in combination with chemotherapy

Angiogenesis and Vascular Morphogenesis. Table 2 Antiangiogenic therapeutic strategies (noncomprehensive list)

Substance	Mechanism
<i>Biological antagonists</i>	
VEGF inhibitors	Humanized neutralizing antibodies, antisense oligonucleotides, siRNA, aptamers
VEGF receptor blockers	Small receptor tyrosine kinase antagonists
VEGF-trap	Inhibition with soluble forms of VEGF-R1, VEGF-R2
$\alpha_v\beta_3$ integrin antagonists	Induce angiogenic endothelial cell apoptosis
<i>Endogenous inhibitors</i>	
Angiostatin	Inhibits EC proliferation, migration, and induces EC apoptosis
Endostatin	Inhibits EC proliferation, migration, and induces EC apoptosis
Vasostatin	Inhibits EC proliferation, adhesion to laminin and induces EC apoptosis
Tumstatin	Inhibits EC proliferation, migration, and induces EC apoptosis
Canstatin	Inhibits EC proliferation, migration, and induces EC apoptosis
Arresten	Inhibits EC proliferation, migration, and induces EC apoptosis
Restin ($\alpha 1$ Collagen IV NC1 fragment)	Inhibits EC proliferation and induces EC apoptosis
Maspin (Mammary serine protease inhibitor)	Induces EC apoptosis
Vascular Endothelial Growth Inhibitor (Protein sharing homology with TNF- α)	Inhibits EC proliferation
Derivative of prolactin	Inhibits EC proliferation and induces EC apoptosis
Proliferin-related protein (PRP) (16 kDa fragment of the prolactin)	Inhibits EC proliferation and induces EC apoptosis
Pigment epithelial-derived factor	Induces EC apoptosis
2-methoxyestradiol (2ME2)	Induces EC apoptosis
IL-12	Modulates angiogenic factors
IL-18	Modulates angiogenic factors
IL-24	Inhibits EC differentiation and migration induced by VEGF and bFGF
Interferons	Inhibits EC proliferation and induces EC apoptosis
NK4 (4-kringle domains of HGF)	Inhibits EC differentiation and migration induced by VEGF
Thrombospondins	Inhibits EC proliferation, migration and induces EC apoptosis by binding to endothelial CD36
<i>Synthetic/semisynthetic inhibitors</i>	
Carboxyamidotriazole	Calcium channel blocker
CM101	Analog of group B streptococcus toxin, binds to tumor endothelium, induces inflammation
Marimastat	Metalloproteinase inhibitor, inhibits endothelial cell invasion
Pentosan polysulfate	Inhibits heparin-binding growth factors
TNP470	Analog of fumagillin, inhibits cell migration and proliferation
Thalidomide	Polycyclic teratogen, antiangiogenic mechanism unknown
<i>Vascular targeting</i>	
Regional TNF- α therapy	Isolated limb perfusion to target in transit metastases
Antibody targeting	Use of mono- and bispecific antibodies to target components of angiogenic blood vessels (e.g., VEGF receptors, endoglin, L19 antigen) to deliver specific angio- and/or tumoricidal activity
Vascular gene therapy	Transfer of dominant-negative receptors or suicide genes under the control of angiogenic endothelial cell specific promoters

for the first line treatment of advanced colorectal tumors. Phase III clinical trials have since been successfully completed for other tumors, including mammary tumors and lung tumors.

VEGF-Trap is a protein-based product candidate designed to bind all forms of VEGF and the related PlGF, and prevents their interaction with cell surface receptors. VEGF Trap is being pursued in phase II

Angiogenesis and Vascular Morphogenesis. Table 3 Antiangiogenic drugs (noncomprehensive list)

Target	Drug
Extracellular matrix	Dalteparin
Endothelial cells	ABT-510
	CNGRC peptide TNF alpha conjugate (NGR-TNF)
	Combretastatin A4 Phosphate
	Dimethylxanthenone Acetic Acide
	Lenalidomide
	LY317615 (Enzastaurin)
	PPI-2458
	soy isoflavone (Genistein; soy protein isolate)
	Tamoxifen Citrate
Activators of angiogenesis	ADH-1 (Exherin™)
	Cetuximab (Erbix™)
	AG-013736
	AMG-706
	Neovastat (AE-941)
	Anti-VEGF Antibody (Bevacizumab; Avastin™; Lucentis™)
	VEGF-Trap
	AZD2171
	Bay 43-9006 (Sorafenib tosylate; Nexavar™)
	BMS-582664
	CHIR-265
	GW786034 (Pazopanib)
	PI-88
	PTK787/ZK 222584 (Vatalanib)
	RAD001 (Everolimus)
	Suramin
	SU11248 (Sunitinib malate; Sutent™)
	SU6668
	XL184
	ZD6474
	ZD1839 (Gefitinib; Iressa)
Endothelial-specific integrin/survival signaling	ATN-161
	EMD 121974 (Cilengitide)
Nonspecific mechanism of action	Celecoxib
	Thalidomide

The table lists drugs with antiangiogenic activity that are in clinical development. Some of the compounds are already clinically approved as specific antiangiogenic drugs (e.g., Bevacizumab [Avastin] or Sunitinib [Sutent]). Others are approved as antiangiogenic as well as antitumorigenic drugs (e.g., Sorafenib [Nexavar]). A third group of drugs is approved as tumorigenic drugs, but is also well established to have antiangiogenic activity (e.g., Cetuximab [Erbix] or Tamoxifen). Most of the listed compounds are in various phases of clinical trials. Given the rapid pace of clinical development, the reader is encouraged to confirm the actual status of a listed compound through an internet search.

studies including advanced ovarian cancer and non-small cell lung adenocarcinoma. In addition, five phase II single-agent studies have been started for relapsed/refractory multiple myeloma, metastatic colorectal cancer, recurrent or metastatic cancer of the urothelium, locally advanced or metastatic gynecological soft tissue sarcomas and recurrent malignant gliomas.

Paralleling the clinical implementation of large molecular weight VEGF inhibitors (antibodies, ligand traps), the first small molecular weight VEGF receptor antagonists have received clinical approval. Sunitinib (Sutent) is a combined VEGF receptor and PDGF receptor inhibitor and thereby blocks angiogenesis and vessel maturation. Sorafenib (Nexavar) targets the VEGF receptors as well as the tumor target c-raf

and acts thereby as a combined antiangiogenic and antitumorigenic drug.

The different VEGF/VEGFR targeting drugs have somewhat varying clinical efficacy. Yet, hitherto approved antiangiogenic drugs in combination with chemotherapy lead on average to an approximately 25% extension of mean life expectancy compared with chemotherapy alone. Depending on the tumor type, this translates in absolute figures to “only” 4.5 to only 9 months. Yet, it needs to be considered that the clinical data are not parametrically distributed, i.e., while some patients respond very beneficially to antiangiogenic treatments, many show little or no clinical response. This reflects the lack of stratifying diagnostic and prognostic procedures that would be necessary to identify those patients that would benefit most from an antiangiogenic intervention (note: “*a targeted therapy requires targeted diagnostic and prognostic procedures!*”)

VEGF and VEGFR blockers specifically interfere with VEGF/VEGFR signaling. Yet, their functional mechanism of action is not well understood. VEGF/VEGFR inhibition blocks angiogenesis and is thereby supposed to starve tumors to death. Yet, more recent work rather suggests that VEGF/VEGFR blockade prunes the immature tumor neovasculature leaving a network of more mature vessels that actually facilitates a more regular blood flow. This “normalization” effect supposedly facilitates better access of chemotherapeutic drugs to the tumor which would explain the empirically determined good synergy of antiangiogenic and chemotherapeutic treatments and the lack of efficacy of antiangiogenic monotherapies. However, the mechanistic dissociation of antiangiogenic tumor starvation versus tumor vessel normalization is subject of intense controversy in the field and focus of much ongoing experimental work.

Inhibitors of the Angiopoietin/Tie-2 and Ephrin/Eph systems are in preclinical development which parallels the biological target validation of these molecules. As vascular assembly, maturation, and homeostasis regulating molecules, therapeutic interference with these molecular systems may hold promise for a number of vascular indications.

Interference with specific cell–cell and cell–matrix adhesion mechanisms is another rapidly advancing approach to therapeutically interfere with angiogenesis. Antagonistic antibodies (Vitaxin) to the integrin heterodimer $\alpha_v\beta_3$ have been shown to act on the blood vessels of tumors but not on the resting organ vasculature. Vitaxin demonstrated some promise in ►Phase II clinical trials.

The growing list of endogenous inhibitors of angiogenesis holds great promise for therapeutic applications (Table 2). Substances most advanced in clinical development include Endostatin, Angiostatin, Interleukin-12, Thrombospondin, and Tumstatin. As

endogenous substances, these molecules have a long half life in the plasma and are, thus, particularly attractive for long-term treatments. Some of them, like Endostatin and Angiostatin, are the proteolytically generated fragments of larger molecules. Endostatin is a 20 kDa C-terminal fragment of collagen XVIII and specifically inhibits endothelial cell proliferation, angiogenesis, and tumor growth. Primary tumors may regress to dormant microscopic lesions. Furthermore, the concept of a dormancy therapy is being extended to Endostatin using cycles of therapy. Angiostatin, a 38 kDa internal domain of plasminogen, is a circulating endogenous protein that supposedly binds ATP synthase on the surface of endothelial cells. It thereby induces endothelial and tumor cell apoptosis, and inhibits endothelial cell migration and tubule formation. Yet, it does not appear to affect growth-factor-induced signal transduction. Angiostatin inhibits matrix-enhanced plasminogen activation, which, in part accounts for its angio-suppressive and anti-invasive properties. Of great interest, Angiostatin is generated by free sulfhydryl donors (e.g., d-penicillamine and captopril) that may partially explain their angio-suppressive properties. Taken together, the clinical development of endogenous angiogenesis inhibitors is proceeding slowly and the results of hitherto pursued clinical trials were not particularly encouraging. It may well emerge that endogenous inhibitors of angiogenesis have little short term interventional therapeutic potential. Yet, they may be very effective preventive drugs which may conceptually actually reflect much better their physiological roles.

Nonspecific Synthetic and Biological Angiogenesis Inhibitors

Systematic screening experiments have identified more than 100 synthetic compounds with potent antiangiogenic activity. The mode of action for most of these molecules is not well understood, but some of the 40 compounds are well advanced in clinical trials (Table 3). The first substance to have entered clinical trials was the Fumagillin-derivative AGM 1470. Fumagillin is an antibiotic which inhibits bFGF- and PDGF-induced endothelial cell proliferation. The mechanism of action of AGM 1470 is poorly understood, but it was shown that it binds and inhibits the metalloprotease methionine aminopeptidase (MetAp-2).

Other antibiotics with antiangiogenic activity are minocycline and herbimycin A. Carboxyamidotriazole (CAI) inhibits the calcium influx into cells and suppresses the proliferation of endothelial cells. It inhibits angiogenesis and metastasis, but it is not an endothelial cell-specific substance. Similarly, the metalloproteinase have long been considered as promising antiangiogenic drugs. Yet, the field is moving slowly following the failure of some MMP inhibitors in advanced clinical trials.

Some natural compounds have also demonstrated antiangiogenic activities. Neovastat (Æ-941) is a naturally occurring (shark cartilage extract), oral agent that shows angio-suppressive and anti-MMP activities in vitro and in the chorioallantoic membrane (CAM) assay. Squalamine (MSI 1256 F), originally derived from the liver of a dogfish shark, is a novel noncytotoxic aminosterol with potent antiangiogenic properties in vivo and in vitro. Squalamine prevents the neovascularization of tumors by suppressing endothelial cell migration and proliferation. Combretastatins are small organic molecules found in the bark of the African bush willow, the *Combretum cafferum*. Combretastatins not only suppress proliferating endothelium, but also specifically target tumor endothelium. The combretastatin A-4 prodrug is a derivative of combretastatin, which is activated by a phosphatase selectively amplified in proliferating endothelial cells. Combretastatin A-4 induces apoptosis in human endothelial cells. In tumor-bearing mice, combretastatin A-4 significantly enhanced the antitumor effects of radiation therapy.

Vascular Targeting

The goal of vascular targeting is to utilize specific molecular determinants of angiogenic endothelium to deliver substances or activities that destroy the vasculature. Unlike antiangiogenic drugs that inhibit the formation of neo-vessels, vascular targeting agents (VTAs) occlude the preexisting blood vessels of tumors to cause tumor cell death from ischemia and extensive hemorrhagic necrosis. VTAs can indirectly kill tumor cells that are resistant to conventional antiproliferative cancer therapies, i.e., cells in areas distant from blood vessels where drug penetration is poor, and hypoxia can lead to radiation and drug resistance. There are broadly two types of VTAs, small molecules and ligand-based, which are grouped together, because they both cause acute vascular shutdown in tumors leading to massive necrosis. The small molecules include tubulin-destabilizing drugs, combretastatin A-4 disodium-phosphate, ZD6126, AVE8062, and Oxi 4503, and the flavonoid, DMXAA. Ligand-based VTAs use antibodies, peptides, or growth factors that bind selectively to tumor versus normal vessels to target tumors with agents that occlude blood vessels. The ligand-based VTAs include fusion proteins (e.g., vascular endothelial growth factor linked to the plant toxin gelonin), immunotoxins (e.g., monoclonal antibodies to endoglin conjugated to ricin A), antibodies linked to cytokines, liposomally encapsulated drugs, and gene therapy approaches.

Proangiogenic Therapies

Antiangiogenesis research has driven the field. Yet, there are a number of indications which may benefit from an induction of angiogenesis, including wound

healing, cardiac ischemia, and peripheral limb ischemia. Various approaches have been taken to therapeutically deliver angiogenic cytokines such as VEGF and FGF-2. These include the local administration of recombinant proteins and gene therapeutic delivery of angiogenic cytokines. Individual cytokine therapy may have limitations as it may be capable of inducing a neovascular response, but it may not induce the growth of a patent neovascular network that is stable for prolonged periods of time. This notion has led to alternative strategies aimed at inducing the complex endogenous angiogenic program and not just a single cytokine. For example, experiments are underway to locally induce hypoxia-inducible factor-1 (HIF-1), a key regulator of the hypoxia response program which is able to control the complex endogenous program of angiogenesis induction. Several angiogenic factors (VEGF-A, VEGF-C, FGF-1, FGF-2 and FGF-4) have been tested in patients with myocardial or limb ischemia.

Direct laser-assisted myocardial revascularization (DMR) is an approved technique in the US, Europe, and parts of Asia to create numerous myocardial channels. This results in the induction of a massive inflammatory reaction, which in turn induces angiogenesis. The other FDA-approved pro-angiogenic therapy is the use of recombinant human platelet-derived growth factor (Regranex) for use in the treatment of diabetic neuropathic foot ulcers.

► Vascular Endothelial Growth Factor

► Matrix Metalloproteinases

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Angioplasty

A percutaneous catheter procedure that inflates a balloon in areas of narrowing (stenosis) in arteries.

► Atherosclerosis

Angiopietins

Angiopietins are growth factor ligands of the receptor tyrosine kinase Tie-2 which are critical regulators of vascular assembly and differentiation.

► Angiogenesis and Vascular Morphogenesis

Angiotensin Converting Enzyme

Synonyms

ACE

Definition

Angiotensin converting enzyme (ACE) is identical to kininase II. It is an essential component of both the renin–angiotensin system and the kallikrein–kinin system. Three different forms of ACE are known. It exists as a membrane bound protein with a molecular weight of 150–180 kD, anchored to the cytoplasmic membrane of endothelial cells and as a circulating protein of similar size. The enzyme consists of two highly homologous lobes with an active site in each lobe. Interestingly, although still of uncertain consequence, the active sites exhibit different catalytic profiles and different affinities for ACE inhibitors. A third, smaller form of ACE (90 kD) with only one active site is expressed in mature germ cells.

The substrate specificity of ACE is low. ACE cleaves a variety of pairs of amino acids from the carboxy-terminal part of several peptide substrates. The conversion of ANG I to ANG II and the degradation of bradykinin to inactive fragments are considered the most important functions of ACE. Both peptides have profound impact on the cardiovascular system and beyond. ACE is thus an important target for ACE inhibitors. These compounds are frequently and efficiently used in the treatment of hypertension and cardiac failure.

► ACE Inhibitors

Angiotensin Converting Enzyme-2 (ACE2)

ACE2 is the closest human homologue of angiotensin converting enzyme is a type I integral membrane

protein that hydrolyses dynorphin A (1–13), apelin-13, apelin-36 and desArg(9) bradykinin.

► Apelins

Angiotensin II Receptor-like 1

► Apelins

Angiotensin Receptors

Angiotensin receptors mediate the effects of angiotensin (ANG) II, the effector peptide of the renin–angiotensin system. Two receptors termed AT1 and AT2 have been characterized in detail. Both receptors belong to the super family of G-protein coupled receptors. Most of the cardiovascular functions of ANG II are mediated through the AT1 receptor. This receptor is usually Gq/11-coupled and its activation leads to intracellular calcium surges and protein kinase C activation. Downstream of the G-protein activation, small GTP-binding proteins such as RAS and RHOA and tyrosine kinase cascades are activated. These include members of the MAP-Kinase and JAK/STAT pathways. Finally transcription factors, such as AP-1, NF- κ -B, and the STATs (signal transducer and activators of transcription) are activated, which initiates the expression of growth-related genes and/or is involved in inflammatory processes. This explains the effects of ANG II on growth, proliferation, and its assumed role in inflammation. The functions of the AT2 receptor are still a matter of debate. The AT2 receptor is probably involved in differentiation processes, inhibits proliferation, and induces apoptosis, and thus may partially counteract some effects of AT1 receptor activation. It is expressed during embryonic development in a tightly controlled manner. In adults it is expressed in adrenal gland and ovary and its expression is induced during inflammatory processes and tissue damage. At present, there are no drugs available, which specifically inhibit or stimulate the AT2 receptor.

► Renin–Angiotensin–Aldosterone System

► ACE Inhibitors

► Nuclear Factor- κ B

Angiotensinogen

- Renin–Angiotensin–Aldosterone System
- ACE Inhibitors

Anion Exchange Resin

Anion exchange resins are basic polymers with a high affinity for anions. Because different anions compete for binding to them, they can be used to sequester anions. Clinically used anion exchange resins such as cholestyramine are used to sequester bile acids in the intestine, thereby preventing their reabsorption. As a consequence, the absorption of exogenous cholesterol is decreased. The accompanying increase in low density lipoprotein (LDL)-receptors leads to the removal of LDL from the blood and, thereby, to a reduction of LDL cholesterol. This effect underlies the use of cholestyramine in the treatment of hyperlipidaemia.

- HMG-CoA-reductase-inhibitors

Ankyrin Repeat

The ankyrin repeat motif is one of the most common protein–protein interaction domains. Ankyrin repeats are modules of about 33 amino acids repeated in tandem. They are found in a large number of proteins with diverse cellular functions such as transcriptional regulators, signal transducers, cell-cycle regulators, and cytoskeletal proteins.

- Nuclear Factor Kappa B
- Adaptor Proteins

Annexins

Annexins form an evolutionary conserved family of Ca^{2+} and phospholipid binding proteins implicated in membrane trafficking and the regulation of Ca^{2+} currents across membranes.

- Ca^{2+} -Binding Proteins

Anomalous Rectifiers

- Inward Rectifier K^{+} Channels

Anorexigenic

Appetite-suppressing. Neuropeptide modulators and gut hormones with anorexigenic effects are α -melanocortin-stimulating hormone (α -MSH), cocaine- and amphetamine-regulated transcript (CART), glucagon-like peptide-1 (GLP-1), leptin, insulin, oxyntomodulin, pancreatic peptide PP, peptide YY and PYY_{3–36}, and others.

- Appetite Control
- Anti-Obesity Drugs

Antacids

Antacids are neutralizing agents. Examples are magnesium hydroxide, magnesium trisylcate and aluminium hydroxide. Prior to the introduction of histamine- H_2 receptor antagonists and proton pump inhibitors, they were the standard drugs for the treatment of duodenal/peptic ulcers. Today their clinical use is limited to the treatment of dyspepsia and the symptomatic relieve for patients with peptic ulcers.

- Histaminergic System
- Proton Pump Inhibitors and Acid Pump Antagonists

Antagonist

Antagonists are natural or synthetic ligands that bind to a receptor but do not cause a downstream signal trasduction process. Antagonists that bind to the same ligand-binding site than the respective agonist act as competitive antagonists. Antagonists (or blockers) that bind to another binding site and exert their pathway-disrupting activity by allosteric effects are termed

noncompetitive antagonists. Antagonists are commonly synthetic or semisynthetic compounds and can be used as drugs in clinical or experimental settings.

- ▶ Drug–Receptor Interaction
- ▶ Transmembrane Signaling
- ▶ G-protein–coupled Receptors
- ▶ Dopamine System
- ▶ Adenosine Receptors
- ▶ Chemokine Receptors
- ▶ Endocannabinoids
- ▶ Nuclear Receptors
- ▶ Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
- ▶ Selective Sex Steroid Receptor Modulators

Anterograde Amnesia

Often referred to as “short-term memory loss”, this form of amnesia results in the inability to transfer new events to long term memory. The sufferer will still be able to recall older memories but will not be able to remember recent events once attention has been switched to something else.

- ▶ Sleep

Anthelmintic Drugs

Anthelmintic drugs are used for the treatment of worm infections. They represent a small but diverse group of drugs with regard to both their chemical structure and their mechanism of action. Like antimicrobial agents they are effective against certain types of worm and ineffective against others. The benzimidazoles (mebendazole, thiabendazole and albendazole) are broad-spectrum agents and the main group of anthelmintics used in the clinic. They induce multiple biochemical changes. However, their main mechanism of action appears to be inhibition of microtubule formation by binding to free parasitic β -tubulin. The spectrum of worms includes nematodes (e.g. the common round worm and the worm causing trichiniasis, *Trichinella spiralis*) and some cestodes. The drug of choice for the treatment of river blindness (caused by *Onchocerca volvulus*) is ivermectin. Trematodes (e.g. *Schistosoma* species causing bilharzia) are sensitive to praziquantel.

Anthracyclins

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Synonyms

Anthraquinones; Antibiotics; Cytotoxics

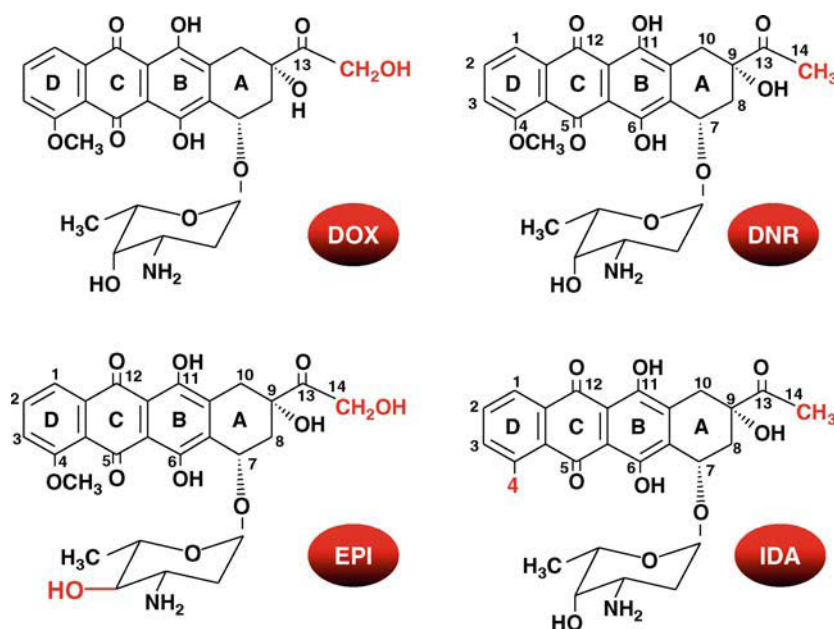
Definition

The ▶anthracyclines represent a broad family of antibiotics that exhibit activity in numerous tumors. The first anthracyclines, doxorubicin (DOX) and daunorubicin (DNR), were isolated from *Streptomyces* var *peucetius*; they were shown to be composed of a tetracyclic ring system with adjacent quinone–hydroquinone moieties, a short side chain with a carbonyl group, and an aminosugar bound to the C-7 of the four-ring system. DOX and DNR only differed in the side chain terminus ($-\text{CH}_2\text{OH}$ in DOX vs. $-\text{CH}_3$ in DNR). Second generation anthracyclines, like epirubicin (EPI) and idarubicin (IDA), were obtained after minor chemical modifications of DOX or DNR, respectively (Fig. 1).

Mechanisms of Action

DOX and other approved anthracyclines have long been known to inhibit ▶topoisomerase II (topo II). This enzyme promotes the formation of double-strand DNA breaks, which are resealed after changing the twisting status of the double helix of DNA. Topo II is highly important to proliferating cells, as the supercoiling of the DNA double helix is modulated according to the cell cycle phase and transcriptional activity. Anthracyclines stabilize a reaction intermediate in which DNA strands are cut and covalently linked to topo II, eventually impeding DNA resealing. Rings B, C, and D are important determinants of anthracycline intercalation into DNA, while ring A and the aminosugar residue play a major role in the formation and stabilization of the ternary complex. Anthracycline- and topo II-mediated DNA damage is followed by growth arrest in G1 and G2 and apoptosis. This is usually, but not always, relayed by p53 and the consequent induction of the WAF1/CIP1 p21 gene product, a strong inhibitor of cyclin-dependent kinases that favor cell cycle progression through the G1 to S transition.

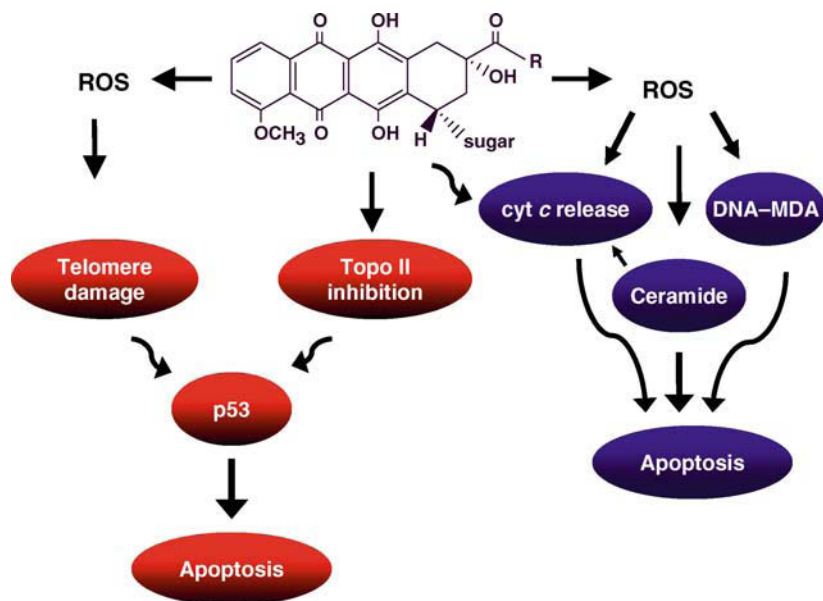
Topo II inhibition remains the most persuasive mechanism to explain the antitumor activity of anthracyclines; accordingly, limited clinical studies showed that tumor



Anthracyclins. Figure 1 Structure of the four main anthracyclins approved for clinical use. The anthracyclins are composed of a tetracyclic ring with adjacent quinone–hydroquinone moieties and a short side chain with a carbonyl group at C-13; an aminosugar is attached by a glycosidic bond to the C-7 of the tetracyclic ring. Doxorubicin (DOX) and daunorubicin (DNR) differ in the side chain terminus ($-\text{CH}_2\text{OH}$ or $-\text{CH}_3$, respectively). Epirubicin (EPI) is obtained after an axial-to-equatorial epimerization of the hydroxyl group at C-4' in the aminosugar. Idarubicin (IDA) is characterized by the absence of the methoxy group at C-4 in ring D.

response and patient's outcome correlated with apoptosis induced by topo II inhibition. Nonetheless, clinically relevant concentrations of anthracyclins were shown to induce apoptosis also through corollary mechanisms that were not bound to topo II or p53; these mechanisms include, among others: (i) the activation of neutral sphingomyelinases, followed by ceramide formation and converse activation of cell death effectors (c-Jun N-terminal kinase) or downregulation of survival pathways (Akt/protein kinase B), (ii) mitochondrial dysfunction, followed by cytochrome *c* (cyt *c*) release and apoptosome formation, (iii) induction of lipid peroxidation and formation of malondialdehyde-DNA adducts, followed by the reduced activity of cyclin E- and cyclin B- associated kinase activities and growth arrest in both p53-proficient and p53-deficient cells, and (iv) inhibition of the [proteasome](#), followed by an accumulation of undegraded ubiquitinated proteins that signal apoptosis [1]. The mechanisms (i–iii) are triggered by [reactive oxygen species](#) (ROS), which are major byproducts of anthracycline metabolism. ROS may also enable anthracyclins to damage and shorten telomeres, long sequences of base repeats that otherwise would delay cell senescence and apoptosis by preventing the degradation and ligation of the end of chromosomes; however, anthracycline-induced telomere damage and dysfunction would be relayed to apoptosis through p53 (Fig. 2).

Anthracycline treatment may be accompanied by the acquisition of a resistance phenotype through a combination of pharmacokinetic and pharmacodynamic mechanisms. On pharmacokinetic grounds, tumor resistance is caused by the reduced accumulation and/or an altered distribution of anthracyclins in tumor cells. A major mechanism of such a kind of resistance rests on the overexpression of drug transporters that belong to the ATP-binding cassette family of proteins and are collectively referred to as ABC proteins (P-glycoprotein/Pgp, [multidrug resistance protein 1/MRP1](#), breast cancer resistance protein/BCRP). For many years, it was thought that ABC proteins could confer resistance to anthracyclins by localizing to the plasma membrane of tumor cells and by mediating drug efflux through a canonical ATP-dependent antiporter mechanism. More recently, [ABC proteins](#) were shown to localize also to the membrane of cytoplasmic acidic organelles like lysosomes, recycling endosomes, and vesicles of the *trans*-Golgi network. Vesicular ABC proteins mediate an [out]→[in] transport of the anthracyclins, which subsequently undergo protonation of their amino residue and remain entrapped in the lumen of the vesicles. The ABC proteins therefore divert anthracyclins away from the nucleus or mitochondria toward pharmacologically unproductive cell sanctuaries like cytoplasmic organelles. Anthracycline-naïve tumor cells do not exhibit such a mechanism of anthracycline



Anthracyclins. Figure 2 Mechanisms of anthracycline-induced apoptosis of tumor cells. ROS, reactive oxygen species; topo II, topoisomerase II; cyt c, cytochrome c.

sequestration, as they usually lack the vesicular expression of ABC proteins or fail to generate a proton gradient across the envelope of cytoplasmic organelles.

On pharmacodynamic grounds, tumor resistance may be caused by such diverse mechanisms as the mutation or redundancy of topo II, the overexpression and preferred nuclear localization of proteasome α -type subunits (leading to an anomalous degradation of topo II), genetic deletion or loss-of-function mutations of p53, overexpression of ROS-detoxifying enzymes, overexpression of Bcl-2 (leading to a diminished cyt c release), etc. However, none of these factors would universally predict the development of anthracycline-resistance in a given tumor or another.

Clinical Use

In spite of their longer than 40 years record of longevity the anthracyclines still rank among the most effective cytotoxics available to the oncologists. DOX and its second generation analogue EPI are essential components of the treatment of breast cancer, childhood solid tumors, soft tissue sarcomas, and aggressive lymphomas. DNR is used to treat acute lymphoblastic or myeloblastic leukemias, while its second generation analogue IDA shows activity also in multiple myeloma, non-Hodgkin's lymphomas, and breast cancer. The broader spectrum of activity of IDA versus DNR is attributed to pharmacokinetic and pharmacodynamic factors such as an increased lipophilicity and cellular uptake, and a stronger stabilization of a ternary anthracycline-topoisomerase II-DNA complex. An additional point of consideration is that DOX, EPI, and DNR must be administered by i.v. boluses, while IDA shows a

nonnegligible bioavailability (~ 10 – 30%) also when administered orally.

Many other anthracyclines were extracted or synthesized over the last two decades, but only few of them eventually progressed toward a robust program of clinical development. A rather benign conclusion is that the novel anthracyclines or related anthraquinones offered only modest advantages over DOX-EPI or DNR-IDA, with the possible exceptions being nemorubicin (for the locoregional treatment of hepatocellular carcinoma), pixantrone (for the second-line treatment of non-Hodgkin's lymphomas), sabarubicin (for the treatment of non-small cell lung cancer, hormone refractory metastatic prostate cancer, platinum- or taxane-resistant ovarian cancer), valrubicin (for the topical treatment of bladder cancer).

As with any other anticancer agent, the clinical use of anthracyclines is limited by hematologic and nonhematologic toxicities. The main nonhematologic toxicity is represented by a life-threatening dilative cardiomyopathy; in the long-term survivors of childhood cancer, cardiomyopathy may surface in a hypertrophic-restrictive form. The precise mechanisms of anthracycline cardiotoxicity remain a matter of debate. The current thinking is that DOX and other anthracyclines may become cardiotoxic after their conversion to ROS or secondary alcohol metabolites. ROS are formed after a one-electron reduction of the quinone moiety and the consequent formation of a semiquinone that reduces oxygen to superoxide anion $O_2^{\cdot-}$, hydrogen peroxide (H_2O_2), hydroxyl radical $\cdot OH$. Due to the lower levels of ROS-detoxifying enzymes in cardiomyocytes as compared with other cell types (and many tumors

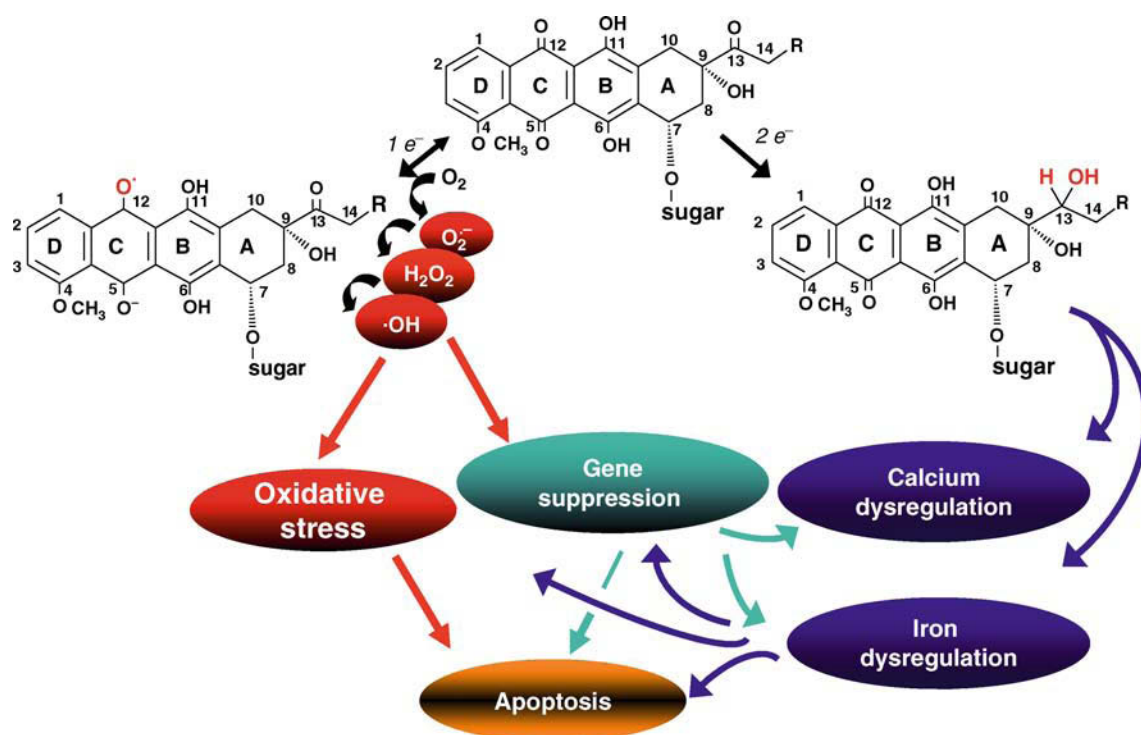
as well), anthracyclins may gradually overrule the cardiac defenses and cause oxidative stress, suppression of cardiac-specific genes, apoptosis. Secondary alcohol metabolites are formed after a two-electron reduction of the side chain carbonyl group; they do not only downregulate cardiac-specific genes, but also inactivate calcium- and iron-handling proteins. The two pathways of toxicity, induced by ROS or secondary alcohol metabolites, share multiple links and feedbacks (Fig. 3).

Anthracycline-induced cardiomyopathy exhibits a well-defined dose-dependence, and may progress toward congestive heart failure (CHF) in the face of medication with β -blockers, calcium antagonists, converting enzyme inhibitors, diuretics. In the case of DOX, the threshold to cardiomyopathy and CHF is currently set at $\sim 500 \text{ mg/m}^2$, but there have been concerns about whether cumulative doses $< 500 \text{ mg/m}^2$ caused nonsymptomatic cardiac dysfunction that could surface at a later time in the form of symptomatic CHF. These concerns are less than speculative if one considers that very many women receive $\sim 300 \text{ mg}$ of DOX/ m^2 for the neoadjuvant (presurgery) or adjuvant (postsurgery) treatment of early breast cancer. The available evidence suggests that these women do develop nonsymptomatic cardiac dysfunction,

usually detected as pathologic or borderline decreases of the [left ventricular ejection fraction](#); however, there is no compelling demonstration that such a dysfunction would eventually progress to symptomatic CHF. Clinically, CHF may occur anytime after the completion of a cumulative anthracycline regimen; children tend to develop cardiomyopathy and CHF at longer times, sometime as late as 15 years after the last of several doses of an anthracycline. This latter observation suggests that changes in the lifestyle, environmental factors, and growth-related hemodynamic challenges may trigger the progression of noncardiotoxic injuries toward symptomatic cardiac dysfunction [2].

Cardiotoxicity may develop at lower than expected cumulative doses of anthracyclins in patients with risk factors like hypertension, preexisting arrhythmias or valvular disease, advanced age, prior irradiation of the mediastinum.

A higher than expected incidence of CHF is also observed in patients treated with DOX and other cytotoxics (e.g., the [taxane](#) paclitaxel) or new generation targeted agents (e.g., the humanized anti-ErbB-2/neu monoclonal antibody trastuzumab) [3]. The cardiotoxic synergism of DOX with taxanes or



Anthracyclins. Figure 3 Mechanisms of anthracycline cardiotoxicity. One-electron ($1 e^-$) reduction of the quinone moiety generates a semiquinone that recycles to the parent quinone by redox coupling with oxygen. The consequent cascade of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot\text{OH}$), induces apoptosis through oxidative stress and gene suppression. Two-electron ($2 e^-$) reduction of the side chain carbonyl group generates a secondary alcohol metabolite that also suppresses cardiac-specific genes and inactivates proteins of calcium and iron homeostasis. These mechanisms share multiple links and feedbacks.

Anthracyclins. Table 1 Strategies for reducing anthracycline cardiotoxicity

Strategy	Rationale	Indications
Substituting EPI for DOX	Lower formation of ROS or secondary alcohol metabolite	Same as those of DOX; combination with drugs that stimulate anthracycline conversion to secondary alcohol metabolite or diminish the cardiac defenses against ROS
Liposomal encapsulation of DOX or DNR	Preferred anthracycline delivery to the tumor	Breast cancer, ovarian cancer, AIDS-related Kaposi's sarcoma, multiple myeloma (pegylated liposomal DOX). Breast cancer (uncoated liposomal DOX). AIDS-related Kaposi's sarcoma, acute myeloblastic leukemia, multiple myeloma, non-Hodg-kin's lymphomas (uncoated liposomal DNR)
Conjugation of DOX with copolymers or peptides	Recognition of DOX by tumor-specific receptors or proteases	Investigational
Coadministration of dexrazoxane	Chelation of iron in the heart, correction of iron dysregulation or mitigation of free radical formation	Approved for use in patients who continue DOX above 300 mg/m ² or require another anthracycline after a prior exposure to 300 mg of DOX/m ²
Substituting slow infusions for 5–10 min boluses	Diminished anthracycline C _{max} and cardiac uptake	At the investigator's discretion (doubtful usefulness in pediatric settings)

EPI, epirubicin; DOX, doxorubicin; ROS, reactive oxygen species; DNR, daunorubicin

trastuzumab has been attributed to anomalous increases of the formation of secondary alcohol metabolites or an interruption of salvage pathways against ROS, respectively. While providing a validation of the mechanistic hypotheses of anthracycline cardiotoxicity, these concepts anticipate that a higher than expected incidence of CHF might well occur, if DOX were combined with other biologic agents that blocked proliferation-survival pathways. Recent studies show that bevacizumab, a humanized antibody against the Vascular Endothelial Growth Factor, synergizes with DOX and causes moderate to severe cardiotoxicity at cumulative doses of 300–420 mg of DOX/m². Other agents that could cause excess cardiotoxicity upon combination with DOX might include sunitinib (inhibitor of the Vascular Endothelial Growth Factor receptor) and lapatinib (inhibitor of the tyrosine kinase domain of both ErbB-1 and ErbB-2 receptors).

The unabated need for anthracyclines in many clinical settings has formed the basis to develop strategies that optimize their activity and/or tolerability. The reversion of the resistance phenotype has been pursued – with a limited success – through the use of first and second generation Pgp inhibitors like verapamil, dexverapamil, cyclosporine A, valspodar, quinidine. Third generation revertants are now available (biricodar, zosuquidar, laniquidar), but the benefits of this strategy remain uncertain [4]. The attenuation of cardiotoxicity is obtained – with a somewhat higher success – through the combination of one or more of the following strategies: (i) substitution of EPI for

DOX, as EPI seems to form fewer amounts of ROS and secondary alcohol metabolite, (ii) encapsulation of anthracyclines in uncoated or pegylated **liposomes** that ensure a good drug delivery to the tumor but not to the heart, (iii) conjugation of anthracyclines with chemical moieties that are selectively recognized by the tumor cells, (iv) coadministration of dexrazoxane, an **iron chelator** that diminishes the disturbances of iron metabolism and free radical formation in the heart, and (v) administration of anthracyclines by slow infusion rather than 5–10 min bolus (Table 1). Pharmacological interventions with antioxidants have also been considered, but the available clinical studies do not attest to an efficacy of this strategy.

► Antineoplastic Agents

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Anthraquinones

► Anthracyclins

Antiarrhythmic Drugs

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Synonyms

Antiarrhythmics

Definition

Antiarrhythmic drugs are substances that affect cardiac ionic channels or receptors, thereby altering the cardiac action potential or its generation or propagation. This results in changes of the spread of activation or the pattern of repolarization. Thereby, these drugs suppress cardiac arrhythmia.

These drugs are traditionally classified according to Vaughan-Williams ([Table 1](#)): (i) sodium channel blockers; slowing the spread of activation (► [antiarrhythmic drugs, class I](#)) with prolongation of the action potential (class IA), with shortening of the action potential (class IB) or without effect on action potential duration (class IC), (ii) β -adrenoceptor antagonists; slowing sinus rhythm and atrioventricular conduction (► [antiarrhythmic drugs, class II](#)), (iii) potassium channel blockers; prolonging the action potential (► [antiarrhythmic drugs, class III](#)), (iv) calcium channel

blockers; mainly slowing atrioventricular conduction (► [antiarrhythmic drugs, class IV](#)), and (not included in this classification) (v) digitalis glycosides, (vi) adenosine, (vii) atropine and (viii) sympathomimetic drugs like orciprenaline.

Mechanism of Action

Basic Considerations

Normal rhythmic activity is the result of the activity of the sinus node generating action potentials that are conducted via the atria to the atrioventricular node, which delays further conduction to the His-Tawara-Purkinje system. From the Purkinje fibres, action potentials propagate to the ventricular myocardium. Arrhythmia means a disturbance of the normal rhythm either resulting in a faster rhythm (tachycardia, still rhythmic) or faster arrhythmia (tachyarrhythmia) or slowed rhythm (bradycardia, bradyarrhythmia).

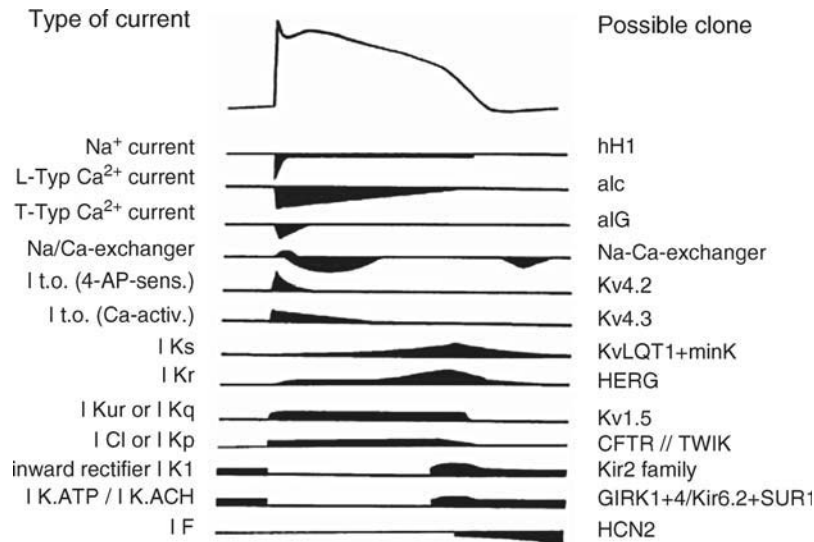
Arrhythmia is either the result of impaired conduction or altered electrical activity. However, in all arrhythmias, conduction and intercellular communication are important since arrhythmia only occurs if the altered electrical activity in one region is transduced to the whole organ.

Antiarrhythmic drugs can either influence electrical activity of the single cell or can interfere with the spread of activation.

In the following, the cardiac action potential is explained ([Fig. 1](#)): An action potential is initiated by depolarization of the plasma membrane due to the pacemaker current (I_f) (carried by K^+ and Na^+ , which can be modulated by acetylcholine and by adenosine) modulated by effects of sympathetic innervation and β -adrenergic activation of Ca^{2+} -influx as well as by acetylcholine- or adenosine-dependent K^+ -channels [in sinus nodal and atrioventricular nodal cells] or to depolarization of the neighbouring cell. Depolarization opens the fast Na^+ channel resulting in a fast depolarization (phase 0 of the action potential). These channels then inactivate and can only be activated if the membrane is hyperpolarized

Antiarrhythmic Drugs. Table 1 Classification of antiarrhythmic drugs according to Vaughan-Williams

Class	Effects	Drugs
I	Block of sodium channel	
I a	With prolongation of action potential	Quinidine, Procainamide, Disopyramide, Ajmaline, Prajmaline
I b	With shortening of action potential	Lidocaine, Mexiletine, Tocainide, Phenytoin, Aprindine
I c	With only little effect on action potential duration	Lorcainide, Flecainide, Propafenone
II	β -adrenoceptor antagonists	Propranolol, Metoprolol and others
III	Block of repolarizing potassium channels, prolongation of action potential	Amiodarone, Dronedarone, Sotalol, Dofetilide, Ibutilide
IV	Block of calcium channels	Verapamil, Diltiazem



Antiarrhythmic Drugs. Figure 1 Transmembrane ionic currents of the cardiac action potential. In the middle of the figure, a typical cardiac action potential is shown as can be obtained from the ventricular myocardium (upper trace). Below, the contribution of the various transmembrane currents is indicated. Currents below the zeroline are inward; currents above the zero line are outward fluxes. In the left column the name of the current is given and in the right column the possible clone; redrawn and modified after [5].

again. This fast upstroke is followed by a short incomplete repolarization (activation of the transient outward rectifier $I_{t.o.}$ carried by K^+ , phase 1). Next, the action potential remains quiet constant for about 50–350 ms (plateau phase, phase 2), which is the result of inward Ca^{2+} current (via L-type Ca^{2+} channels) and simultaneous activation of the repolarizing potassium current, the delayed rectifier I_K (which has three components: rapid, $I_{K,r}$, ultrarapid, $I_{K,ur}$, slow component, $I_{K,s}$). This is followed by complete **repolarization** of the membrane to about –80 mV via activation of the delayed rectifier (phase 3), while the Ca^{2+} channels close during this phase. During an action potential it is not possible to elicit a second action potential since the fast Na^+ channel is inactivated. This period is called refractory period [4].

Not all cells in the heart express the fast sodium channel. Thus, sinus nodal and atrioventricular nodal cells lack the fast Na^+ channel and instead generate their action potentials via opening of Ca^{2+} channels. This is the basis for their sensitivity to Ca^{2+} antagonists.

Action potential propagation along the fibre is mainly dependent on the Na^+ channel availability, which is a function of the resting membrane potential. Propagation from cell to cell is realized via intercellular gap junction channels. These channels can be regulated by a number of stimuli. Thus, e.g. low pH, high $[Ca^{2+}]_i$ or low $[ATP]_i$ result in a closure of these channels leading to conduction disturbances and arrhythmias in myocardial infarction. Moreover, the density of these channels can be regulated by e.g. chronic adrenergic stimulation, angiotensin-II or endothelin and has been

found to be altered in arrhythmias such as atrial fibrillation or arrhythmogenic conditions like cardiac hypertrophy.

There are several basic mechanisms of arrhythmia:

1. A single cell or group of cells capable of a pacemaker potential may generate extrastimuli (enhanced automaticity).
2. A cell may generate oscillating afterpotentials which reach the threshold for activation of the Na^+ channel (triggered activity).
3. A cell generates late afterdepolarizations (typically induced by catecholamines or digitalis) following a complete repolarization that may elicit an action potential.
4. A cell may produce early afterdepolarizations that are depolarization during incomplete repolarization. This is possible if the action potential is considerably prolonged. This is the typical mechanism for elicitation of Torsade de Pointes arrhythmia, a typical complication of class III antiarrhythmics and many other drugs.
5. Furthermore, under certain conditions (e.g. local unidirectional block) it is possible that the activation wavefront is delayed and encounters areas already repolarized. This may result in a circulating wavefront (= reentrant circuit **reentrant arrhythmia**), from which centrifugal activation waves originate and elicit life-threatening ventricular fibrillation.
6. Block of propagation may occur in the specific conduction system leading to bradyarrhythmia

(sinusatrial block, atrioventricular block, intraventricular bundle block).

Antiarrhythmic treatment is based upon modulation of the ionic currents mentioned above. A principal problem with this therapy is that the electrophysiology of all cells is targeted and not specifically the arrhythmogenic focus. As a consequence, all antiarrhythmics acting at transmembrane ionic channels possess a risk for elicitation of arrhythmia (= proarrhythmic risk).

Molecular Mechanism of Action

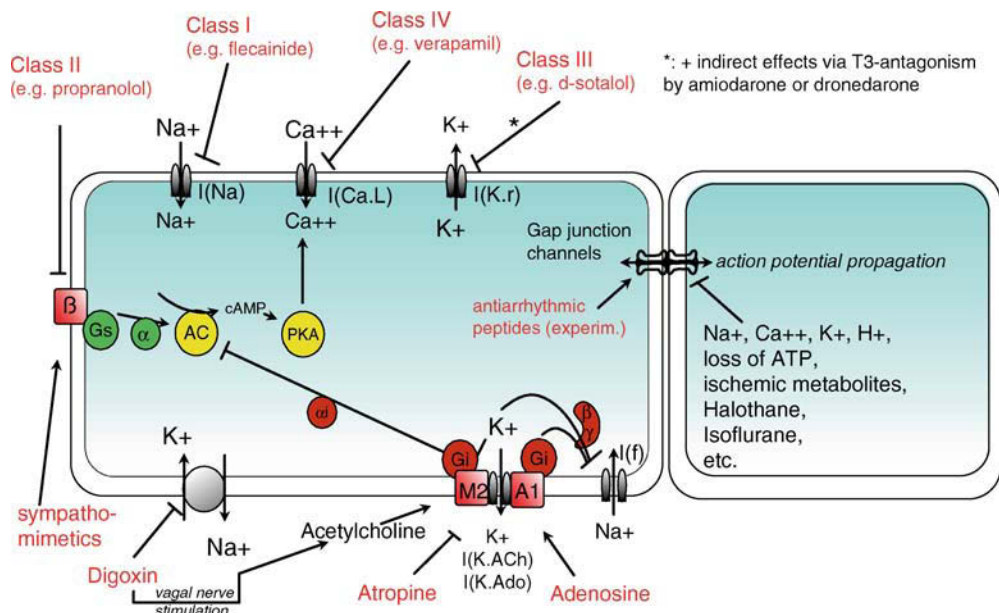
Class I, III and IV antiarrhythmics bind to and block transmembrane ionic channels (Fig. 2). Class I antiarrhythmics block the fast Na^+ channel. This channel switches from a resting state to an open state, and then time- and voltage-dependently inactivates (inactivated state) (Fig. 3). The block of this channel by an antiarrhythmic drug is a **state-dependent block**: a class I compound like lidocaine enters the channel in its open state and binds to the inactivated state, altering the kinetics of recovery from inactivation. If the channel switches to its resting state, the affinity for lidocaine is less and the drug dissociates from the channel. This is the basis for the use-dependence of block: the kinetics of dissociation determines the interval after which a subsequent action potential is not influenced. That means that an action potential early after the foregoing action potential will be suppressed while another after a long interval will not be altered.

Drugs with fast dissociation will only suppress high frequency arrhythmia (high use-dependence). Drugs with a long dissociation time constant will suppress action potentials at normal frequency as well. Class IB drugs exhibit the shortest time constant (0.2–0.4 s; highest use-dependence), while class IC drugs have the longest dissociation time constant (2–250 s; no use-dependence). Class IA antiarrhythmics show an intermediate dissociation time constant (5–50 s).

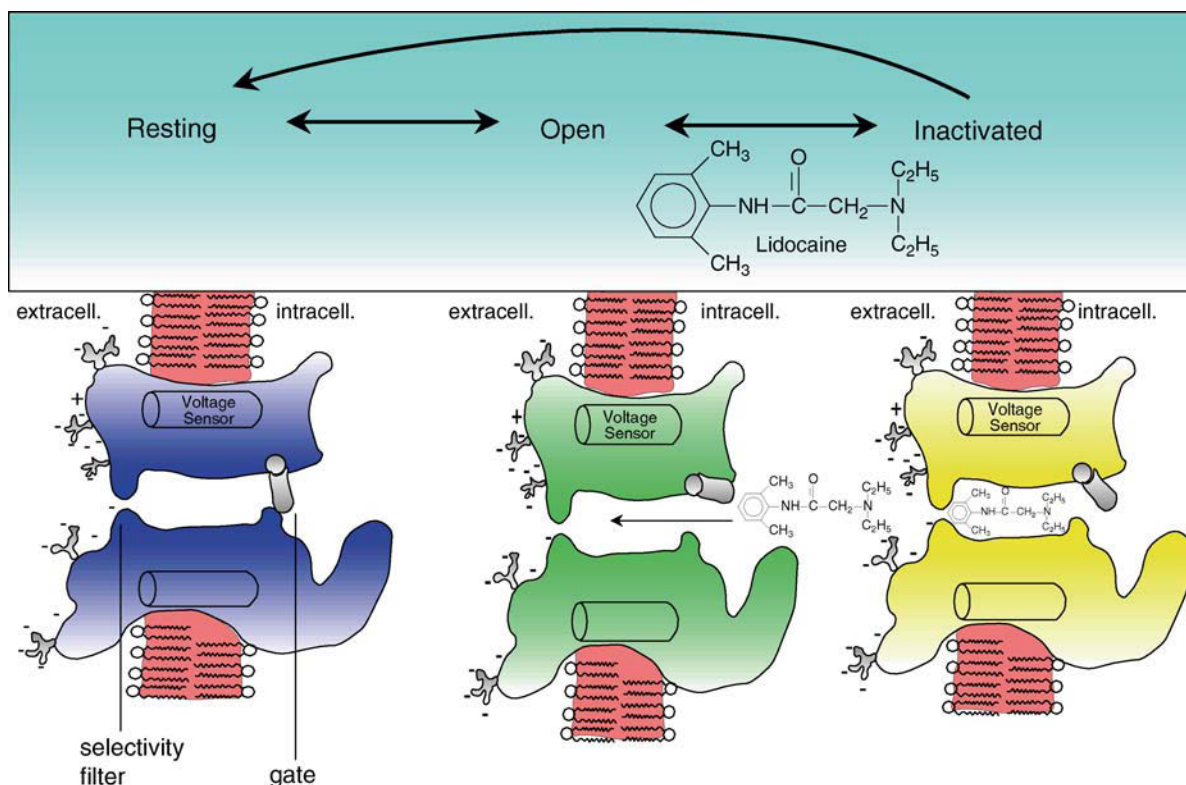
Class I drugs (Na^+ channel blockers) suppress action potential amplitude, reduce fast depolarization (upstroke) velocity and propagation velocity, prolong total refractory period and reduce automaticity. These drugs have no or little influence on slow Ca^{2+} carried action potentials (AV- or sinus nodal cells). All these drugs prolong the ventricular QRS complex, exert negative inotropic effects and a strong proarrhythmic effect especially in patients with structural heart disease and, thus, cannot be used in patients after myocardial infarction.

Besides the class I-typical proarrhythmic risk class IA antiarrhythmics possess a marked proarrhythmic risk for the induction of **torsade de pointes arrhythmia** (life-threatening polymorphic ventricular tachycardia observed with most action potential prolonging drugs).

Quinidine, the classical class IA drug, binds to the open state of the Na^+ channel, and prolongs the action potential by block of the delayed rectifier. In higher concentrations, L-type Ca^{2+} channels are inhibited. Quinidine exerts antimuscarinic effects, thereby accelerating AV-nodal



Antiarrhythmic Drugs. Figure 2 Targets of the various antiarrhythmic drugs. The K^+ channel, regulated by acetylcholine (via M_2 receptors) or adenosine (via A_1 receptors), plays a role only in supraventricular tissues. The action potential which is generated in one cell propagates to the neighbouring cell via the gap junction channel.



Antiarrhythmic Drugs. Figure 3 Binding of class I antiarrhythmic drugs to the cardiac sodium channel. Summary of the modulated receptor hypothesis as an explanation of state-dependent block of Na^+ channels by local anaesthetics such as lidocaine. The Na^+ channel switches between resting and open and inactivated state. On the extracellular side, a selectivity filter controls the ions passing through the channel. On the intracellular side, the inactivation gate can close the channel. A class I antiarrhythmic drug (lidocaine) enters the channel during its open state and binds with its lipophilic moiety to the inactivated state, the hydrophilic part of the molecule extending into the water-filled channel pore blocking it.

conduction and antagonising α -adrenergic effects, which may lead to accelerated AV-conduction and faster ventricular rhythms in situations as atrial fibrillation or atrial flutter. Typical untoward effects include vomiting, diarrhoea, allergies, immunological and haematological effects and hepatitis.

Ajmaline (intravenously only) and its orally applicable propyl-substituted prodrug prajmaline are classified as class IA drugs, but due to their long dissociation time constant can also be considered as class IC compounds.

Further class IA drugs include the open state blockers procainamide and disopyramide with electrophysiological effects similar to those of quinidine; procainamide lacks the antimuscarinic and antiadrenergic effects. Characteristic side effects of procainamide are hypotension and immunological disorders.

Class IB drugs like lidocaine, phenytoin or mexiletine preferentially bind to the inactivated state. Lidocaine, a local anaesthetic, can be used intravenously for antiarrhythmic treatment. It is one of the classical drugs used in emergency medicine for the

treatment of ventricular fibrillation. The side effects of lidocaine are typical for local anaesthetics including dizziness, tremor, nystagmus, seizures or nausea.

The antiepileptic drug phenytoin, an orally available class IB antiarrhythmic, is mainly effective in digitalis-induced arrhythmias. This drug exhibits non-linear pharmacokinetics and a number of side effects including neuropathy, gingival hyperplasia, hepatitis, immunological disorders and suppression of white blood cells.

Class IC antiarrhythmic drugs such as flecainide or propafenone block the Na^+ channel (open state; propafenone: open and inactivated state) with a very long dissociation time constant so that they alter normal action potential propagation. Flecainide increased mortality of patients recovering from myocardial infarction due to its proarrhythmic effects (CAST study). Action potential is shortened in Purkinje fibres but is prolonged in the ventricles.

Propafenone possesses β -adrenoceptor antagonistic effects due to its structural similarity to propranolol.

In high concentrations it blocks calcium channels and, thus, exerts prominent negative inotropic effects. Its adverse effects include proarrhythmic effects, worsening of heart failure and (due to β -adrenoceptor blockade) bradycardia and bronchospasm.

Class II drugs are classical β -adrenoceptor antagonists such as propranolol, atenolol, metoprolol or the short-acting substance esmolol. These drugs reduce sinus rate, exert negative inotropic effects and slow atrioventricular conduction. Automaticity, membrane responsiveness and effective refractory period of Purkinje fibres are also reduced. The typical extracardiac side effects are due to β -adrenoceptor blockade in other organs and include bronchospasm, hypoglycemia, increase in peripheral vascular resistance, depressions, nausea and impotence.

Class III antiarrhythmic drugs block the repolarizing K^+ channel thereby prolonging the action potential duration and lengthen the refractory period. The classical class III antiarrhythmic compounds like sotalol block the rapid component of the delayed rectifier, I_{Kr} . However, at higher heart rates the repolarization is mainly carried by the slow component I_{Ks} and, thus, the action potential prolonging effect of these agents is progressively reduced with increasing heart rate (inverse use-dependence). Class III antiarrhythmics can induce early afterdepolarizations and torsade de pointes, a polymorphic ventricular tachycardia associated with excessive QT prolongation, which can degenerate into ventricular fibrillation.

d,l-Sotalol is a racemate of l-sotalol, a β -adrenoceptor antagonist, and d-sotalol, an inhibitor of both I_{Kr} and I_{Ks} . This substance exhibits a strong inverse use-dependence. Regarding the beneficial antiarrhythmic effects, studies with only l-sotalol showed that the racemate is superior. However, due to β -adrenoceptor blockade d,l-sotalol can induce bronchoconstriction, increase in peripheral vascular resistance, negative inotropy, depressions, hypoglycemia and bronchospasm. A serious side effect is the induction of torsade de pointes arrhythmia (ca. 3%).

Amiodarone blocks several ionic channels; besides predominant blockade of I_{Kr} and I_{Ks} , as well as I_{K1} , I_{KACH} , I_{sus} , I_f , I_{Na} , I_{CaL} , I_{CaT} is inhibited. Moreover, amiodarone acts as an antagonist at both α - and β -adrenoceptors. Inverse use-dependence is less than with sotalol. The action potential prolonging effect is slowly developing (steady state after 2–5 months). From today's understanding the molecular mechanism includes the generation of an inactive triiodothyronine isomere thus acting as a functional T3-antagonist affecting the T3-dependent gene activation, which seems to explain the slowly developing prolongation of the action potential. A small acute prolongation of the action potential is probably dependent on the formation of the metabolite desethylamiodarone. Side effects

include defects of vision, corneal depositions, neurological disorders, pigmentation, photosensibilization, torsade de pointes arrhythmia (but less than 1%) and AV-block. Moreover, alterations of thyroid function with both hypo- (due to the inactive T3-isomere) and hyperthyroidism (due to the iodine content of the drug) and lung fibrosis are observed.

Since alterations of thyroid function by amiodarone are related to the iodine substitution of the drug, the iodine-free derivative dronedarone has been developed with similar electrophysiological effects as amiodarone. It seems to act also as a T3-antagonist, but does not provoke hyperthyroidism [1].

Newly developed class III drugs comprise dofetilide, a specific I_{Kr} blocker, and ibutilide, which blocks I_{Kr} and activates the slow I_{Na} . Both drugs lack hemodynamic side effects. These drugs are scheduled for the treatment of atrial fibrillation and atrial flutter. As with class III drugs, they can induce torsade de pointes arrhythmia.

Class IV antiarrhythmic drugs (Ca^{2+} entry blockers) inhibit L-type Ca^{2+} channel. For antiarrhythmic purposes, only those Ca^{2+} channel antagonists are used with higher affinity to the heart (i.e. phenylalkylamines like verapamil, gallopamil and D600, or benzothiazepine derivatives like diltiazem) than to the vasculature (as nifedipine or other 1,4-dihydropyridines, which therefore do not belong to the class IV antiarrhythmics). Class IV antiarrhythmic drugs like verapamil or diltiazem exert the strongest electrophysiological effects on sinus and atrioventricular node, since in sinus and AV-nodal cells I_{Na} is not expressed but action potentials are carried by Ca^{2+} . They reduce sinus rate, slow atrioventricular conduction, prolong refractory period of the AV node and exert a strong negative inotropic and vasodilator effect. Ventricular electrophysiology is only slightly affected, but force of contraction is markedly reduced.

Verapamil is a phenylalkylamine which blocks L-type Ca^{2+} channels in a use-dependent manner. The drug binds to the inactivated state of the channel. Diltiazem is a benzothiazepine derivative with a profile of action most similar to that of verapamil.

Other Antiarrhythmic Compounds

► **Cardiac glycosides** exert parasympathomimetic effects by activation of vagal nerves leading to slowing of sinus rate and predominantly to prolongation of atrioventricular conduction time. This latter action is used for the control of the ventricular response frequency in the treatment of atrial fibrillation with digitalis (frequency control therapy of atrial fibrillation). In that indication it can be combined with verapamil or β -adrenoceptor antagonists.

Adenosine activates the atrial A_1 -adenosine receptor, which opens the I_{KAdo} channel leading to

hyperpolarization, slowing of spontaneous depolarization, reduces sinus rate and atrioventricular conduction [3]. The drug has to be administered intravenously. It produces a short cardiac arrest and is used for termination of atrioventricular reentrant tachycardia. Due to its extremely short half-life time (0.6–1.5 s) the effects are only transient. Since adenosine is also a potent vasorelaxant, it may produce pronounced hypotension, and in patients suffering from asthma bronchospasm. The latter effects are transduced via other adenosine receptors. This is the basis for the development of a new A_1 -selective agonist, tecadenoson.

The antimuscarinic drug atropine, and its derivative ipratropiumbromide, can also be used for antiarrhythmic treatment. Muscarinic receptors (M_2 subtype) are mainly present in supraventricular tissue and in the AV node. They inhibit adenylylcyclase via G_i proteins and thereby reduce intracellular cAMP. On the other hand, activation of the M_2 receptor leads to opening of hyperpolarizing $I_{K,ACH}$ and inhibits the pacemaker current I_f probably via the $\beta\gamma$ -subunit of the G_i protein associated with this receptor. The results are hyperpolarization and slower spontaneous depolarization. Muscarinic receptor antagonists like atropine lead to increased heart rate and accelerated atrioventricular conduction. There are no or only slight effects on the ventricular electrophysiology.

Intravenous administration of magnesium sulfate (1–5 g) is used for the termination of torsade de pointes arrhythmia. The underlying electrophysiological mechanism is not well understood. It includes changes of the current–voltage relationship of I_{K1} and Ca^{2+} channel blockade.

Clinical Use

Clinical uses of antiarrhythmics have been restricted after CAST [2] due to their proarrhythmic risk, and preference is given to electrophysiological methods.

Supraventricular bradycardia is treated by implantation of a pacemaker device or has been treated pharmacologically with atropine. Supraventricular paroxysmal tachycardia is treated with ajmaline or prajmaline. Supraventricular tachyarrhythmias or AV reentrant arrhythmia typically can be terminated using adenosine.

The risk of atrial flutter is a 2:1 transmission to the ventricles generating a high ventricular rate. The therapeutic goal is to reduce transmission to 3:1 or 4:1 by administration of either β -adrenoceptor antagonists, Ca^{2+} channel blockers or amiodarone. Quinidine must not be used in this arrhythmia, since it accelerates AV-conduction due to its vagolytic effect.

The most common arrhythmia in humans is atrial fibrillation. Because of the lack of rhythmic atrial activation, irregular ventricular rhythms and thromboembolism result. There are two possible therapeutic goals:

control of heart rate or return to sinus rhythm. For frequency (heart rate) control, β -adrenoceptor antagonists, Ca^{2+} channel blockers and digitalis can be used. For conversion to sinus rhythm, electrophysiological ablation is the therapy of choice. Alternatively, a pharmacological attempt with class IA drugs or class IC drugs can be made. Thereafter, relapse to atrial fibrillation has to be prevented by amiodarone or β -adrenoceptor antagonists. A recent concept is the pill-in-the-pocket concept which means that the patient takes just one dose of oral flecainide (or similarly acting drugs) when experiencing a relapse to atrial fibrillation. It is necessary to include oral anticoagulant therapy with phenprocoumon or warfarin in the treatment strategy of chronic atrial fibrillation to prevent from stroke, which is the main risk of a patient suffering from atrial fibrillation.

Atrioventricular block in general is treated by implantation of an electrical pacemaker. A pharmacological alternative (although no longer used today) was atropine. However, atropine can be used for bridging the time between the onset of symptoms and the definitive implantation of a pacemaker.

Ventricular extrasystoles are treated only if they may degenerate into life-threatening arrhythmia. In milder forms the proarrhythmic risk of the drugs overshadows their benefits. In such cases β -adrenoceptor antagonists may be attempted. For the treatment of ventricular extrasystoles, such as series or runs of extrasystoles, amiodarone or sotalol are used. In the absence of structural heart disease, class I antiarrhythmic drugs can be considered an alternative. However, they may not be administered during the post-infarction period.

Ventricular fibrillation should be terminated by electrical defibrillation. Alternatively, lidocaine can be injected intravenously. In cases with lower frequency, ventricular tachyarrhythmia class I drugs such as ajmaline, flecainide or propafenone are more effective as a result of the use-dependence of lidocaine. For prophylaxis treatment, amiodarone or sotalol may be helpful or the implantation of a cardioverter-defibrillator system. Acute amiodarone (i.v.; in higher doses) can also terminate ventricular tachyarrhythmias. This action, however, seems to be mediated by its I_{Na} -blocking side effects and not (or less) by its class III like effects.

Torsade de pointes arrhythmia can be terminated by intravenous (not oral) administration of large doses of magnesium.

- ▶ K^+ Channels
- ▶ Voltage-dependent Na^+ Channels
- ▶ Voltage-dependent Ca^{2+} Channels
- ▶ β -Adrenergic System
- ▶ Cardiac Glycosides

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Antiarrhythmic Drugs Class I

Antiarrhythmic drugs are antagonists of the fast Na^+ channel, which slow the propagation of the cardiac action potential. Class I drugs suppress the fast upstroke of the action potential.

► Antiarrhythmic Drugs

Antiarrhythmic Drugs Class II

Class II antiarrhythmic drugs are β -adrenoceptor antagonists such as propranolol, metoprolol or atenolol. β -adrenoceptor antagonists slow sinus rate and atrioventricular conduction and exert negative inotropic effects.

► Antiarrhythmic Drugs

Antiarrhythmic Drugs Class III

Class III antiarrhythmic drugs are drugs which act as K^+ channel antagonists and result in action potential prolongation without effect on the upstroke of the action potential.

► Antiarrhythmic Drugs

Antiarrhythmic Drugs Class IV

Class IV antiarrhythmic drugs are Ca^{2+} channel blockers, which predominantly slow sinus rate and atrioventricular conduction and thus are used in the treatment of supraventricular tachyarrhythmias. These drugs exert a pronounced negative inotropic effect.

► Antiarrhythmic Drugs

Antibiotic Resistance

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Definition

From the mechanistic point of view three basic principles of microbial resistance to drugs are known: inactivation of the drug, alteration of the target, and reduced drug accumulation at the target site. However, several variations on these themes are known.

Basic Mechanisms

The phenomenon of bacterial resistance to antibiotics was already known by the pioneers of the era of antibiotics, like Paul Ehrlich, who coined the term “selective toxicity” as the basic principle of antimicrobial therapeutics, as well as Gerhard Domagk, the inventor of the sulfonamide drugs, and Sir Alexander Fleming, the discoverer of the penicillins. When penicillin G was introduced into clinical practice in 1944, as many as 5% of the isolates of *Staphylococcus aureus* were resistant to penicillin, while 5 years later the percentage was 50%.

That bacterial resistance predates the era of clinical use of antibiotics by several hundred millions of years is the recent result of genomic sequence data mining from antibiotic-producing microorganisms. These are supposed to be the “inventors” of antibiotic resistance genes which they had “developed” to protect themselves from the lethal action of their own antibiotics [4].

Sharing these resistance genes with other bacteria, including human pathogens, by ► [gene transfer mechanisms](#) has resulted in a wide spread and in the adaption of these foreign gene sequences to the genetic codon usage of the respective pathogen. This is the preferred

strategy to provide bacteria with resistance determinants to naturally occurring antibiotics [1].

In addition, due to their the short generation times resulting in high cell densities within a short time, bacteria can easily acquire mutations in genes encoding antibiotic targets. The usually haploid bacterial genome assures that resistant variants of these targets are phenotypically expressed and, thus, mediate resistance. With this strategy bacteria are able to respond to novel chemical structures of synthetic antibacterials.

Over 4 decades, between 1960 and 2000, the development of new antibiotics used well characterized basic structures for partial synthetic modifications, primarily to overcome resistance by increasing the pharmacodynamic properties and, secondarily, to improve the pharmacokinetic profile of older compounds. However, bacteria rapidly responded by acquiring additional genetic alterations either as mutations or by accumulating resistance genes as part of mobile genetic elements (► **integrons**) on transferable ► **resistance plasmids**.

Inactivation of the Drug

The inactivation of a drug is an enzymatic process which results either in the cleavage of a chemical bond, e.g. by proteases or esterases, or the formation of a new chemical bond, e.g. by transferring phosphate (phosphotransferase), acetate (acetyltransferase), or nucleotidylate (nucleotidyltransferase) moieties to a functional group of the antibiotic. The cleavage causes the loss of function of the antibiotic. Chemical modification can eliminate a specific molecular interaction between drug and target or can alter the physico-chemical properties of a drug, e.g. electrical charge, that has an impact on the ability of a drug to penetrate the bacterial membrane(s).

Proteases

An example for proteases are the β -lactamases that hydrolyse a peptide bond in the essential β -lactam ring of penicillins, cephalosporins, carbapenems and monobactams and, thereby, irreversibly inactivate the drug. β -lactamases share this mechanism with the ► **penicillin binding proteins (PBPs)**, which are essential enzymes catalyzing the biosynthesis of the bacterial cell wall. In contrast to the PBPs which irreversibly bind β -lactams to the active site serine, the analogous complex of the drug with β -lactamases is rapidly hydrolyzed regenerating the enzyme for inactivation of additional β -lactam molecules.

According to their genetic relationship and their biochemical mechanism of action β -lactamases are divided into enzymes of the serine-protease type containing an active-site serine (molecular class A, C, and D enzymes) and those of the metallo-protease type (molecular class B enzymes), which contain a complex bound zinc ion.

The further classification of the over 400 different enzymes described so far into different subclasses is based upon different parameters, like substrate profile, molecular mass, isoelectric point, stability against β -lactamase inhibitors, localization of the encoding gene on a plasmid or on the chromosome (Fig. 1). According to this classification the group 1 enzymes are cephalosporinases of Gram-negative pathogens (most enterobacteria and ► *Pseudomonas aeruginosa*) which usually are chromosomally encoded. In many bacteria, like several enterobacteria (Citrobacter, Enterobacter, Serratia, Providencia, Morganella) their expression can transiently be induced in the presence of β -lactams, however, this induction is not of clinical relevance. Instead, the constitutive overexpression of the genes due to a mutation inactivating a genetic repressor of the β -lactamase will result in a high level of resistance to cephalosporins and penicillins, but not carbapenems. Enzyme inhibitors, like clavulanic acid are not active against these enzymes.

The group 2 contains the greatest number of enzymes showing the broadest spectrum of substrates. The majority of these enzymes belongs to the plasmid-encoded families TEM, SHV, CTX-M and OXA. Each family consists of one or a few progenitor enzymes with narrow substrate profile, e.g. TEM-1, TEM-2, and TEM-13, and several descendents which have acquired mutations resulting in the extension of the substrate profile (► **extended spectrum β -lactamases, ESBL**). This is accomplished either by widening the binding pocket or by introducing amino acids with side chains allowing for the stabilization of the drug-enzyme interaction. These group 2 enzymes are usually inhibited by clavulanic acid, however, some variants exist, which are resistant to the action of β -lactamase inhibitors (IR- β -lactamase), but have lost their broad substrate spectrum.

For the CTX-M family which has been rapidly growing during the last three years genomic data have identified the genome of *Kluyvera* species as the genetic source of progenitor enzymes (e.g. encoding CTX-M1, CTX-M2 or CTX-M9).

► **Metallo-enzymes** belonging to group 3 naturally show a very broad substrate spectrum including all β -lactams except monobactams and are not inhibited by clavulanic acid, but by complexing agents, like EDTA. This can only be exploited for diagnostic purposes.

Esterases

Hydrolysis of macrolides by products of the *ere* genes detected in enterobacteria is only of scientific interest, while esterases VGB-A and VGB-B encoded by the *vgb* type genes mediate clinically relevant resistance in staphylococci to the B compound (quinupristin) of the streptogramin combination quinupristin-dalfopristin.

Classification of β -lactamases - by
molecular and functional features

Mole- cular class	Ambler group	Enzymatic aktivty	Localisation and way of distribution of gene	Examples
1	C	Cephalosporinase	Chromosome, Gram- negatives	AmpC
2a	A,D	Penicillinase	<i>S.aureus</i> , <i>P. aeruginosa</i>	BlaZ
2b	A	"broad spectrum"	Plasmid, Gram-negatives	TEM-1
2be	A	TEM-1,-2; SHV-1 "extended spectrum"	Plasmid, Gram-negatives Chromosome, <i>K. oxytoca</i>	SHV-1 TEM-3 to 161 SHV-2 to 104 CTX-M1 to n
2br	A	"inhibitor resistant"	Plasmid, Gram-negatives	TEM-30 to 41 SHV-10
2c	A	Carbenicillinase PSE; CARB	Plasmid, Gram-negatives	CARB-1
2d	D	Cloxacillinase	Plasmid, Gram-negatives	OXA type
2e		Cephalosporinase	Chromosome, <i>P. vulgaris</i>	CepA
2f	A	Carbapenemase, ceph-, pen-ase	Chromosome, <i>E. cloacae</i> , <i>S.marcescens</i>	SME-1, MNC-A, KPC-1
3	B	Metalloenzym	Chromosome, <i>B. cereus</i>	L1, CfiA, IMP-1, VIM-1
4	?	Penicillinase	Chromosome, <i>P. cepacia</i>	

Antibiotic Resistance. Figure 1 According to Bush, Jacoby and Medeiros [2] four molecular classes of β -lactamases can be discriminated based upon biochemical and molecular features. Classes 1, 2, and 4 included serine-proteases, while metallo enzymes are included in class 3. The substrate spectrum varies between different subclasses and the corresponding genes can be part of an R-plasmid leading to a wider distribution or are encoded chromosomally in cells of specific species.

Acetyltransferases

The preferred substrates of acetyltransferases are amino-groups of antibiotics, like chloramphenicol, streptogramin derivatives, and the various aminoglycosides. The modification is believed to block a functional group involved in the drug-target-interaction. All acetyltransferases use acetyl-coenzyme A as cofactor.

The major mechanism of resistance to chloramphenicol is mediated by the chloramphenicol acetyltransferases (CAT enzymes) which transfer one or two acetyl groups to one molecule of chloramphenicol. While the CAT enzymes share a common mechanism, different molecular classes can be discriminated. The corresponding genes are frequently located on integron-like structures and are widely distributed among Gram-negative and – positive bacteria.

Resistance to the A compound of streptogramins is mediated by products of the *vat* genes (virginiamycin acetyltransferase), of which at least five molecular classes are known (*vatA-E*). These genes are located on plasmids of *Enterococcus faecium* and frequently in combination with a gene encoding a streptogramin hydrolase (*vga/vgb*) in staphylococci.

Enzymes transferring an acetyl moiety to one specific of several amino-groups of the aminocyclitol–aminoglycoside antibiotics (e.g. gentamicin, amikacin, kanamycin) are called aminoglycoside acetyltransferases

(AAC). These enzymes are also widely distributed among Gram-positive and Gram-negative pathogens and are classified according to the position of the amino group modified (e.g. AAC3, AAC6'). They are further subdivided into different molecular classes based upon substrate profile (e.g. AAC6'-I, AAC6'-II) and amino acid sequence homology (e.g. AAC6'-IIa, AAC6'-IIb). A summary of relevant aminoglycoside modifying enzymes (AMEs) mediating resistance to clinically used aminoglycosides is given by Shaw et al [3].

Nucleotidyltransferases, Phosphotransferases

Beside AAC enzymes two different enzyme classes, nucleotidyltransferases (ANT enzymes), and phosphotransferases (APH enzymes) modify the hydroxyl groups of aminocyclitol–aminoglycoside antibiotics.

Alteration of the Target

Target alterations usually result in the reduction of the affinity for a drug. This strategy enables the bacteria to respond to alterations in the selective pressure due to, e.g. novel antibiotics within a short period of time. Genetic basis for a target alteration are either point mutations in the respective target gene or acquisition of a DNA fragment containing information for a an altered less susceptible target. Alternatively, targets can be modified enzymatically.

Target Alteration by Point Mutations

The major mechanism of resistance to fluoroquinolones is the acquisition of point mutations in the genes *gyrA* and *parC* encoding the respective A subunits of both targets, ►topoisomerases II and IV. Only a few codons are affected which are located in the so-called quinolone resistance-determining region (QRDR) between ala-67 and gln-106 (*E. coli* numbering of *gyrA*). To achieve a high level of resistance, at least two *gyrA* and one *parC* mutations are required.

Other examples include rifampin resistance due to mutations in the *rpoB* gene encoding the β -subunit of RNA polymerase, or oxazolidinone resistance due to a G2576T mutation in the gene for the 23S rRNA as central part of the 50S large ribosomal subunit. Macrolide resistance is based upon the alteration of nucleotide A2058 by a point mutation.

Target Alteration by Enzymatic Modification

An alternative strategy to modify nucleotide A2058 of the 23S rRNA is used by the majority of pathogens: the production of a specific methyltransferase which couples one or two methyl groups to the N6 amino group thereby reducing the affinity of macrolides to their target. Since this residue is also involved in the binding of other antibiotics, like streptogramin B and lincosamine, this mechanism is termed MLS_B type [4].

Another more sophisticated mechanism of enzymatic alteration of the target is the transfer of the complete regulon encoding a D-alanyl-D-lactate-ligase, like VanA, as basis for transferable resistance to glycopeptide resistance. In cooperation with some accessory factors the structure of the basic units of the bacterial cell wall, the disaccharide-pentapeptide with a C-terminal D-alanine, is transformed into a variant carrying a C-terminal D-lactate which prevents the binding of glycopeptide antibiotics at physiological concentrations and, thus, causes glycopeptide resistance. Such regulons reside on transposons allowing for the rapid exchange of the genetic information between different enterococcal species and even to staphylococci.

Target Modification by Acquisition of Genes Encoding Resistant Target

In contrast to macrolides, the targets of β -lactams, the penicillin binding proteins (PBPs) require several mutations in order to become resistant while simultaneously maintaining their viable function as cell wall transpeptidases/transglycosidases. Thus, in order to achieve clinically relevant resistance *Streptococcus pneumoniae* uses a unique strategy to rapidly accumulate several point mutations. Due to its natural competence for transformation during respiratory tract

infections *S. pneumoniae* cells can acquire and insert into their chromosomes genetic material from closely related species, like viridans group streptococci. Since these cells carry genes for PBPs with reduced sensitivity to β -lactams due to the presence of several genetic variations, transformation/recombination generate so-called ►mosaic genes encoding a β -lactam-resistant variant protein, PBPX.

Staphylococcus aureus cells can acquire large DNA fragments containing the *mecA* gene which encodes a complete new penicillin binding protein 2A (PBP 2A), as part of a transposon. PBP2A can substitute the natural set of penicillin-sensitive PBPs thereby mediating a complete cross resistance to all β -lactam antibiotics.

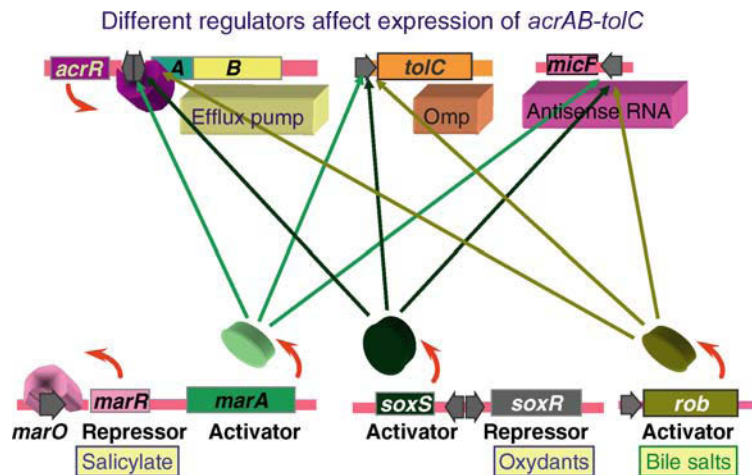
Reduced Drug Accumulation at Target Site

Uptake of nutrients from the environment, release of cell signalling molecules and virulence factors as well as disposal of toxic compounds are essential for metabolic active microorganisms. In contrast to Gram-positive bacteria having a cytoplasmic membrane only, Gram-negative bacteria are surrounded by an additional outer membrane, which forms a strict barrier between the intracellular space and the environment. While lipophilic compounds can directly penetrate the membranes' lipid bilayers, hydrophilic molecules require transmembrane proteins forming water-filled pores for passive diffusion into the cell or energy-driven transmembrane pumps for active efflux.

Two mechanisms are operating alone or in concert to minimize the antibiotic concentration at the intracellular target site: Downregulation of the expression of the pore proteins, also called porins, and upregulation of one or a set of several unspecific efflux pumps. However, the impact of these mechanisms on the resistance is low, since due to the essential function of porins for uptake of nutrients their reduction is limited and to avoid disturbances of membrane integrity due to extensive overproduction of *mdr* efflux pumps these are subjected a strict regulation.

Resistance Due to Multiple-Drug Resistance (*mdr*) Efflux

Currently, five different molecular classes of *mdr* efflux pumps are known [5]. While pumps of the the ATP-binding cassette (ABC) transporter superfamily are driven by ATP hydrolysis, the other four superfamilies called resistance-nodulation-division (RND), major facilitator superfamily (MFS), multidrug and toxic compound extrusion (MATE), and small multidrug resistance transporter (SMR) are driven by the proton-motive force across the cytoplasmic membrane. Usually a single pump protein is located within the cytoplasmic membrane. However, the RND-type pumps which are restricted to Gram-negative bacteria consist of two additional components, a periplasmic membrane fusion protein (MFP) which connects the efflux pump to an outer



Antibiotic Resistance. Figure 2 The major *mdr* efflux pump of *Escherichia coli* belongs to the RND superfamily and consists of the pump AcrB, the membrane fusion protein AcrA, and the porin TolC. The expression of the genes *acrAB* is under control of the local repressor AcrR, while *tolC* and *acrAB* are additionally regulated by several global transcriptional activators, like MarA induced by salicylate, SoxS derepressed by oxidants, and by Rob directly activated by bile salts.

membrane porin. This architecture allows the disposition of the antibiotics outside the cell (Fig. 2). Efflux is due to an enzymatic activity and therefore saturable.

The combined intrinsic activities of different efflux pumps play a major role for the intrinsic resistance of Gram-negative bacteria to macrolides and oxazolidinones as well as to the intrinsic resistance of *Pseudomonas aeruginosa* against a broad range of disinfectants and antibiotics.

Acquired resistance has been observed by constitutive upregulation of *mdr* efflux pump expression due to a mutation inactivating a respective repressor or inducibly, caused by molecules transiently inactivating repressor molecules upon binding. Depending upon the substrate spectra of the respective subset of efflux pumps upregulated, a multiple drug resistance (*mdr*) phenotype is expressed, which in combination with a specific resistance mechanism can contribute to a clinically relevant level of resistance.

Resistance to Tetracyclines Due to Specific Efflux Pumps

For some naturally occurring antibiotics, like chlormphenicol and tetracyclines, specific drug efflux pumps have been detected in antibiotic-producing bacteria. The tetracycline specific efflux pumps have been detected in many bacterial pathogens and are the major mechanism of resistance to these drugs. Several of the corresponding structural genes are inducibly expressed: In the presence of tetracyclines in the growth medium a few drug molecules enter the cell by diffusion, bind to the pump repressor and, thus, induce tet efflux pump expression.

► Quinolones

► Microbial Resistance to Drugs

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Antibiotics

Originally, the term antibiotics referred to substances produced by microorganisms that suppressed the growth of other organisms. Today, the term antibiotics often includes synthetic antimicrobial agents.

► β -Lactam Antibiotics

► Ribosomal Protein Synthesis Inhibitors

► Microbial Resistance to Drugs

► β -Lactam Antibiotics

- Ribosomal Protein Synthesis Inhibitors
- Anthracyclins

Antibodies

Antibodies are involved in the humoral immune response. They recognize foreign substances (antigens) and trigger immune responses by the host. For the former, they possess interaction sites for a specific antigen. These interaction sites (Fab portions) are highly variable between antibodies produced by different clones of B cells. For the latter, they possess a constant region (Fc portion). Engineered antibodies are increasingly used for the treatment of human diseases.

- Immune Defense
- Humanized Monoclonal Antibodies

Antibodies to Cyclic-citrullinated Peptides (Anti-CCPs)

It has long been known that antiperinuclear factor and antikeratin antibodies have high specificity for rheumatoid arthritis. Both these antibodies recognize epidermal fillagrin, a protein involved in the cornification of the epidermis. The amino acid target of these antibodies is citrulline, which is derived from the amino acid arginine after peptide translation under the influence of the enzyme peptidyl arginine deiminase. Antibodies directed at cyclic citrullinated peptides have a similar sensitivity to rheumatoid factor, but higher specificity.

- Rheumatoid Arthritis

Antibody-dependent Cellular Cytotoxicity (ADCC)

A mechanism of cell-mediated immunity whereby an effector cell of the immune system actively lyses a target cell that has been bound by specific antibodies. The typical ADCC involves activation of natural killer (NK) cells and is dependent on the recognition of

antibody-coated cells by Fc receptors on the surface of the NK cell. The Fc receptors recognize the Fc (constant) portion of antibodies such as IgG, which bind to the surface of a target cells. Following binding NK cells release cytokines such as IFN- γ and cytotoxic granules containing perforin and granzymes that enter the target cell and promote cell death by triggering apoptosis.

- Immune Defence
- Interferons
- Tyrosine Kinase Inhibitors

Anticancer Drugs

- Antineoplastic Agents

Anticoagulants

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Synonyms

Oral anticoagulants, usually coumarin derivatives (e.g., warfarin, phenprocoumon); heparin, either unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH); danaparoid (heparinoid); fondaparinux (indirect factor Xa-inhibiting pentasaccharide); drotrecogin α (recombinant human activated protein C [APC]); direct thrombin inhibitors (DTIs), including hirudin derivatives (e.g., lepirudin, desirudin) or analogues (e.g., bivalirudin) and small molecule active site inhibitors (e.g., argatroban, ximelagatran)

Definition

Anticoagulants inhibit ►coagulation by preventing ►thrombin generation and, ultimately, fibrin formation [1]. They represent one of the two major classes of antithrombotic drugs, the other being antiplatelet agents. Anticoagulants are widely used to treat and prevent thrombosis involving arteries, veins, and intracardiac chambers.

In general, arterial thrombi are platelet-rich (“white clots”) and form at ruptured atherosclerotic plaques, leading to intraluminal occlusion of arteries that can result in end-organ injury (e.g., myocardial infarction, stroke). In contrast, venous thrombi consist mainly of fibrin and red blood cells (“red clots”), and usually form in low-flow veins of the limbs, producing deep vein thrombosis (DVT); the major threat to life results when lower extremity (and, occasionally, upper extremity) venous thrombi embolize via the right heart chambers into the pulmonary arteries, i.e., pulmonary embolism (PE).

Mechanism of Action Overview of Coagulation

Figure 1 shows a simplified scheme of the coagulation cascade. Coagulation is usually triggered physiologically when ►tissue factor (TF), usually found in extravascular sites, binds to circulating factor VII(a) following vessel injury. TF/VII(a) complexes activate factor X, generating factor Xa. Factor Xa, together with a cofactor (factor Va), forms “prothrombinase” on phospholipid surfaces on activated platelets. Prothrombinase generates the key procoagulant enzyme, thrombin (factor IIa), from prothrombin (factor II). Various positive feedback loops help to convert a small procoagulant stimulus into a thrombin burst. For example, TF/VII(a) complexes also activate factor IX to IXa, which acts with a cofactor (VIIIa) to form the

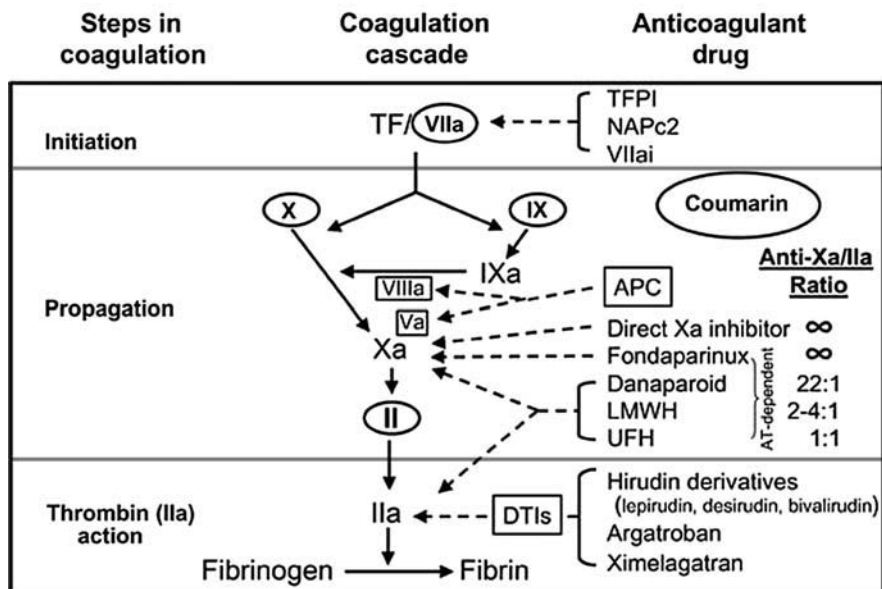
“tenase” complex that activates factor X to Xa. Other positive feedback loops initiated by thrombin include activation of factors V to Va, VIII to VIIIa and XI to XIa (not shown in Fig. 1).

Coagulation is regulated by three major inhibitory systems. (i) Antithrombin (AT, formerly, antithrombin III) inhibits circulating thrombin, Xa, IXa, XIa and TF/VII(a). However, AT does not inhibit thrombin bound to fibrin (“clot-bound thrombin”) or surface-bound Xa. (ii) The protein C natural anticoagulant pathway is triggered when thrombin binds to a receptor (thrombomodulin, TM) on endothelial cell surfaces: TM-bound thrombin activates protein C to APC, which together with a cofactor (protein S) degrades factors Va and VIIIa, thus downregulating thrombin generation in the TM-rich microcirculation. (iii) Tissue factor pathway inhibitor (TFPI) binds to and inhibits factor Xa; subsequently, TFPI/Xa complexes inhibit VII(a) within VII(a)/TF.

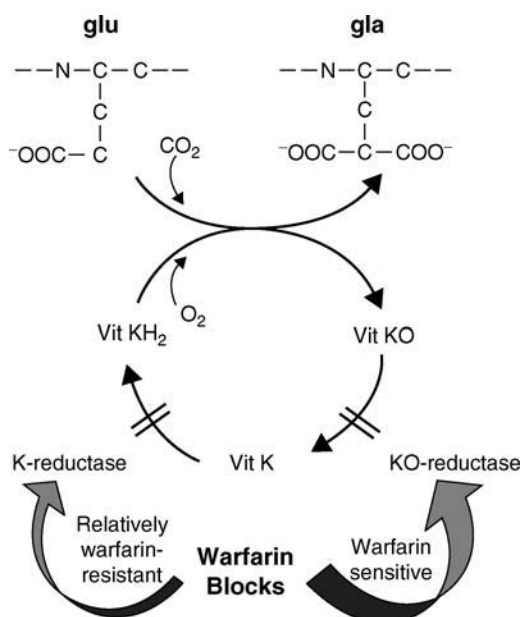
The most commonly used anticoagulants – coumarins and heparins – interfere with various steps, involving “propagation” of the coagulation cascade. Several newer agents inhibit thrombin directly. Drugs that inhibit initiation of coagulation are under investigation.

Oral Anticoagulants (Coumarins)

Most oral anticoagulants are coumarin derivatives that act via ►vitamin K antagonism ([2]; Fig. 2). Vitamin K



Anticoagulants. Figure 1 Effects of anticoagulants on the coagulation cascade. Coumarin agents alter the synthesis of four procoagulant zymogens (VII, X, IX, II), shown within circles. The other anticoagulants affect various coagulation factors (dotted arrows). Abbreviations: APC, activated protein C; AT, antithrombin; DTIs, direct thrombin inhibitors; LMWH, low-molecular-weight heparin; NAPc2, nematode anticoagulant protein; TF, tissue factor; TFPI, tissue factor pathway inhibitor; UFH, unfractionated heparin; VIIai, active site-blocked VIIa. (Modified from [1], with permission from Chest.)



Anticoagulants. Figure 2 Coumarin (Warfarin) and the vitamin K cycle. Abbreviations: glu, glutamate; gla, γ-carboxyglutamate. (Modified from [2], with permission from Chest.)

is required for posttranslational modification of certain glutamate (glu) residues in four procoagulant factors (II, VII, IX, X). Addition of a carboxyl group (COO^-) to each glu residue (to form γ-carboxyglutamate, or gla, residues) causes these vitamin K-dependent factors to become functional ►zymogens (proenzymes), as they now can bind to phospholipid surfaces via Ca^{2+} -recognizing gla regions. Protein C and protein S are vitamin K-dependent anticoagulant factors.

The two most widely used coumarins are warfarin (US, Canada, and UK) and phenprocoumon (continental Europe). The long half-life (60 h) of prothrombin means that coumarin cannot achieve therapeutic anticoagulation for at least 5 days following initiation. Thus, for patients with acute thrombosis, oral anticoagulants are usually started only when the patient is receiving a rapidly active agent, usually UFH or LMWH.

Disadvantages of oral anticoagulants include a narrow therapeutic index (bleeding risk), their highly variable dose–response relation (ongoing need for monitoring), embryopathy (if administered during the first trimester of pregnancy), and potential to induce microvascular thrombosis (coumarin necrosis syndromes).

Maintenance doses widely vary among patients (e.g., from 1 to 20 mg/day for warfarin), and are influenced by diet (variable vitamin K intake) and medications that affect coumarin metabolism (decreased drug clearance: e.g., cotrimoxazole, amiodarone, erythromycin; increased clearance: e.g., barbiturates, carbamazepine, rifampin). Thus, regular monitoring is needed

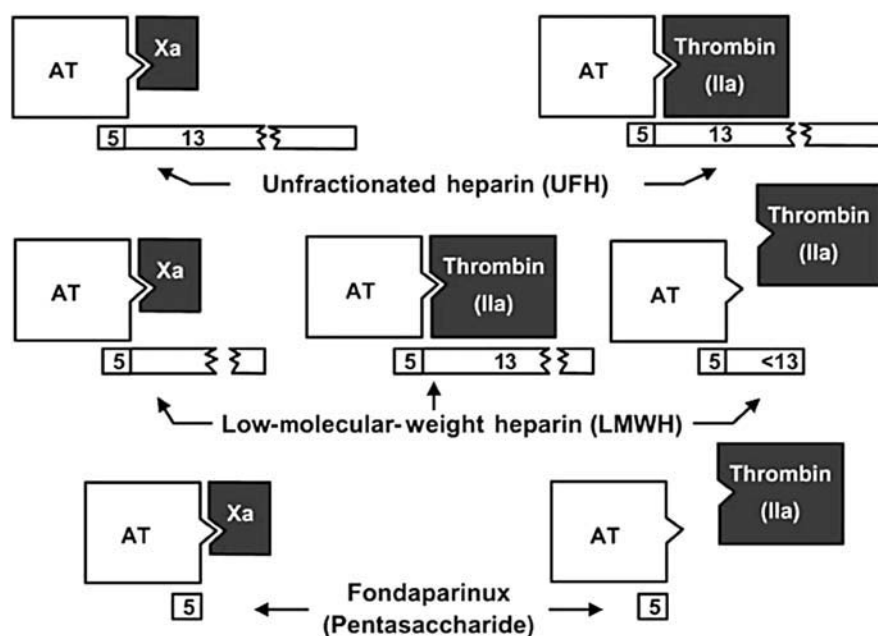
even during long-term maintenance therapy. This is performed using the ►prothrombin time (PT), which is usually expressed as the ►international normalized ratio (INR).

AT-dependent Anticoagulants: Heparins, Danaparoid, and Fondaparinux

Heparin is a highly sulfated ►glycosaminoglycan [3]. Usually obtained from pig intestine or beef lung, UFH contains polymer varying from 3,000 to 30,000 Da (mean, 15,000 Da; range, 10–90 monosaccharide units). Chemical or enzymatic methods can be used to make LMWH preparations that vary from 1,000 to 10,000 Da (mean, 4,500 Da; range, 3–30 monosaccharide units). A specific five saccharide sequence (“AT-binding pentasaccharide”) present within up to one third of UFH chains binds to AT, greatly increasing the efficiency of AT to inactivate thrombin, Xa, IXa, XIa, and TF/VII(a). AT is most efficient at inactivating thrombin and Xa, as shown by higher second-order rate constants ($8,900$ and $2,500 \text{ M}^{-1}\text{s}^{-1}$, respectively) compared with values of 300–450 for VII(a)/TF, IXa and XIa, respectively). Catalysis by UFH increases AT-mediated inhibition 1,000-fold.

Besides containing the specific AT-binding pentasaccharide sequence, heparin molecules must be at least 18 monosaccharide units long to bind to both AT and thrombin; in contrast, AT bound to any pentasaccharide-containing heparin – even with a chain length <18 monosaccharide units – will inhibit factor Xa. Thus, whereas UFH catalyzes inhibition of thrombin and Xa equally well, LMWH preferentially inhibits factor Xa (usual anti-Xa/anti-IIa ratio, 2–4:1) (Fig. 3). LMWH preparations (e.g., ardeparin [Normiflo], dalteparin [Fragmin], enoxaparin [Lovenox], reviparin [Clivarin], tinzaparin [Innohep]) differ in both jurisdictional availability and composition, and cannot be assumed to be interchangeable.

The ►activated partial thromboplastin time (aPTT) is usually used to monitor the anticoagulant effect of UFH, with the target aPTT level corresponding to an anti-factor Xa level of 0.35–0.70 U/mL (i.e., a ratio of patient/control aPTT of 1.5–2.5 for many aPTT reagents). However, prolongation of the aPTT is not sufficiently great to permit monitoring of LMWH therapy by this test. Nevertheless, since the shorter LMWH polymers have less nonspecific binding to plasma proteins, LMWH anticoagulation is quite predictable. Thus, weight-adjusted LMWH dosing without monitoring is standard practice. Particularly during inflammation (high levels of UFH-binding proteins), high doses of UFH may be needed to prolong the aPTT and anti-factor Xa levels into the therapeutic range (heparin “resistance”). Anticoagulant monitoring of LMWH using anti-factor Xa levels may be needed in renal failure as LMWH accumulates.



Anticoagulants. Figure 3 Relative effects of UFH, LMWH, and fondaparinux on AT-mediated inhibition of factor Xa and thrombin (IIa). Whereas UFH catalyzes inhibition of Xa and thrombin equally well, only LMWH chains of 18 saccharide units or longer catalyze thrombin inhibition; thus, the anti-Xa/anti-IIa ratio of LMWH preparations ranges from 2:1 to 4:1. In contrast, fondaparinux exclusively inhibits Xa. (Modified from [3], with permission from Chest.)

Danaparoid (Orgaran; mean MW, 6,000 Da) is a mixture of nonheparin glycosaminoglycans derived from pig gut (dermatan sulfate, heparan sulfate, chondroitin sulfate). The anti-Xa/anti-IIa ratio (22:1) is even greater than seen with LMWH. The anti-IIa effect may be mediated in part by dermatan sulfate, which catalyzes thrombin inhibition by heparin cofactor II.

Fondaparinux, the factor Xa-binding pentasaccharide (Arixtra, MW 1,728 Da), is prepared synthetically, unlike UFH, LMWH and danaparoid, which are obtained from animal sources. Despite only inactivating free factor Xa, clinical trials indicate that fondaparinux is an effective antithrombotic agent, both for venous thromboembolism prophylaxis and treatment, as well as for acute coronary syndrome and ST elevation myocardial infarction [4].

In addition to the AT-dependent agents discussed above, various direct Xa inhibitors (e.g., tick anticoagulant peptide, antistatin, DX-9065a) are undergoing clinical testing. Unlike fondaparinux, these drugs also inhibit surface-bound Xa within prothrombinase.

Activated Protein C

Protein C is a vitamin K-dependent natural anticoagulant activated by thrombin to form APC in the presence of the endothelial receptor, TM. APC proteolyzes factors Va and VIIIa, thus downregulating thrombin generation. APC may also have anti-inflammatory

properties, as recombinant human APC (drotrecogin α , Xigris) reduces mortality in **septicemia**. Nonactivated protein C concentrates, prepared from pooled plasma, are also available for use in patients with congenital or acquired protein C deficiency.

Direct Thrombin Inhibitors

There are two major classes of DTIs: hirudin derivatives and small molecule active site inhibitors. Hirudin is a 65-amino acid polypeptide produced by the medicinal leech, which binds irreversibly and with high affinity to both the active site and exosite I (fibrinogen binding site) regions of thrombin, resulting in stable non-covalent hirudin–thrombin complexes (dissociation constant, $\sim 10^{-14}$ M). Hirudin binds to both circulating and clot-bound thrombin. Lepirudin (Refludan, MW $\sim 7,000$ Da) and desirudin (Revasc) closely resemble hirudin. In contrast, bivalirudin (Angiomax, MW $\sim 2,180$ Da) is a 20-amino acid oligopeptide consisting of the active site and exosite I regions of hirudin connected by a short “spacer.” All three agents are obtained by recombinant technology.

Two small molecule DTIs are argatroban (Novastan, MW 527 Da) and the oral thrombin inhibitor, ximelagatran (Exanta, MW 474 Da) Ximelagatran is an inactive pro-drug; after absorption, it is metabolized to the active DTI, melagatran [MW 430 Da]. Concerns regarding hepatotoxicity have prevented (xi)melagatran

from successful regulatory and marketplace adoption. In general, levels of DTIs are monitored indirectly using the ►aPTT (usual target therapeutic range, about 1.5–2.5-times baseline aPTT). The DTIs prolong the INR in the order: argatroban > bivalirudin > lepirudin.

Factor VII(a)/Tissue Factor Pathway Inhibitors

Recombinant TFPI (tifacogin) directly inhibits VII(a)/TF complexes. Unlike recombinant APC, TFPI did not reduce mortality in clinical trials of septicemia. Recombinant nematode anticoagulant protein (NAPc2) is a small hookworm protein that binds to a noncatalytic site on both X and Xa, thus inhibiting VII(a)/TF. The half-life of NAPc2 is long (48 h), resembling that of factor X. Active site-blocked VIIa (factor VIIai) achieves an anticoagulant effect by competing with VII(a) for binding to TF.

Clinical Use (Including Side Effects)

Both UFH and LMWH are used when rapid anticoagulation is needed, such as acute venous thromboembolism (DVT and/or PE), acute coronary insufficiency (acute myocardial infarction or unstable angina), or for ►percutaneous coronary intervention (PCI). UFH is also used for intraoperative anticoagulation during cardiac surgery employing cardiopulmonary bypass (CPB) as well as during vascular surgery. ►Protamine sulfate is used to reverse UFH anticoagulation after heart surgery.

Treatment of DVT or PE consists of therapeutic-dose heparin, given as intravenous UFH or subcutaneous LMWH or fondaparinux with overlapping oral anticoagulation. Until the early 1990s, UFH was usually given alone for 5 days, followed by at least 5 days of UFH/coumarin overlap, then several months of coumarin anticoagulation. Now, coumarin is often started within 24 h of initiating UFH or LMWH. Duration of coumarin typically ranges from as low as 6 to 8 weeks (small calf-vein DVT in a transient prothrombotic situation, such as postsurgery) to indefinite (multiple prior DVTs complicating a chronic hypercoagulability state). Often, treatment of DVT or PE employing LMWH followed by oral anticoagulants occurs exclusively in an outpatient setting.

Prevention of DVT and PE (antithrombotic prophylaxis) is another common indication for UFH, LMWH or coumarin, especially following surgery or immobilizing trauma. Fondaparinux is approved for prevention of DVT and PE after hip and knee surgery, and following abdominal surgery.

Coumarin is also widely used for long-term anticoagulation in chronic atrial fibrillation (particularly to avoid cardioembolic strokes), to prevent DVT or PE in patients with chronic hypercoagulability (e.g., congenital AT or protein C deficiency), or to prevent

atherothrombosis in patients with atherosclerosis. Coumarin is often unsuccessful in patients with hypercoagulability states, such as immune heparin-induced thrombocytopenia or cancer-associated disseminated intravascular coagulation. In contrast, LMWH therapy is often appropriate for patients with cancer-associated hypercoagulability or to prevent or treat thrombosis during pregnancy.

Danaparoid, lepirudin, and argatroban are important options for rapid anticoagulation when UFH or LMWH are contraindicated (e.g., heparin-induced thrombocytopenia). Desirudin is approved in some jurisdictions for antithrombotic prophylaxis after hip replacement surgery. Bivalirudin is an alternative to heparin for anticoagulation during ►PCI; both bivalirudin and argatroban are approved for anticoagulation during PCI in patients in whom heparin is contraindicated because of acute or previous heparin-induced thrombocytopenia.

Side Effects

Bleeding is the most common adverse effect of anticoagulants [1–3] and is often associated with overdosing. When bleeding occurs during anticoagulation within the target therapeutic range, factors such as recent surgery or gastrointestinal lesions often coexist. For bleeding caused by coumarin overdosing, vitamin K will reverse anticoagulation beginning at least 4 h after administration. More urgent reversal can be achieved by coagulation factor replacement, using plasma or prothrombin complex concentrates. Rapid reversal of UFH is achieved by ►protamine sulfate (1 mg protamine for 100 U heparin). However, only about 60% of the anticoagulant effect of LMWH is neutralized by protamine. Specific antidotes are not available for danaparoid, fondaparinux, DTIs, or inhibitors of the VII(a)/TF pathway. Thus, careful patient selection and anticoagulant monitoring are usually needed to reduce bleeding risk with these newer agents.

Unusual adverse effects sometimes occur with coumarin [2] or heparin [5]. For example, coumarin-induced skin necrosis is a rare complication of oral anticoagulants characterized by (sub)dermal microvascular thrombosis that usually begins 3–6 days after commencing coumarin. Typically, central tissue sites such as the breast, abdomen, and thigh are affected. Congenital abnormalities of the protein C natural anticoagulant pathway are implicated in some patients. A related syndrome of microvascular thrombosis can lead to limb gangrene in some patients treated with oral anticoagulants during heparin-induced ►thrombocytopenia and hypercoagulability. This syndrome of coumarin-induced venous limb gangrene has been linked to severe protein C depletion during use of warfarin to treat DVT, complicated by metastatic cancer or heparin-induced thrombocytopenia.

As many as 3–5% of postoperative patients who receive UFH for 2 weeks develop heparin-induced thrombocytopenia, also known as HIT. This hypercoagulable state is caused by IgG antibodies that recognize complexes between heparin and platelet factor 4 (a platelet α -granule protein). Paradoxically, patients with HIT remain at high risk for thrombosis, even when heparin is discontinued or heparin is replaced with coumarin. To avoid coumarin-induced venous gangrene, alternative anticoagulants such as danaparoid, lepirudin, or argatroban should be given, and coumarin delayed until thrombocytopenia has resolved. Vitamin K should be given to reverse coumarin if HIT is diagnosed after warfarin or another vitamin K antagonist has been given. Long-term UFH treatment can cause ►osteoporosis, likely because heparin both decreases bone formation by osteoblasts and increases bone resorption by osteoclasts. Both HIT and osteoporosis are less likely to occur with LMWH.

Coumarins are generally contraindicated for use during pregnancy, particularly the first trimester. This is because γ -carboxyglutamate (gla), containing proteins are found in bone. Thus, pharmacologic vitamin K antagonism can cause embryopathy (chondrodysplasia punctata). LMWH is an attractive option for many pregnant women who require anticoagulation.

- Antiplatelet Drugs
- Coagulation/Thrombosis
- Fibrinolytics

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Anticonvulsants

- Antiepileptic Drugs

Antidepressant Drugs

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Synonyms

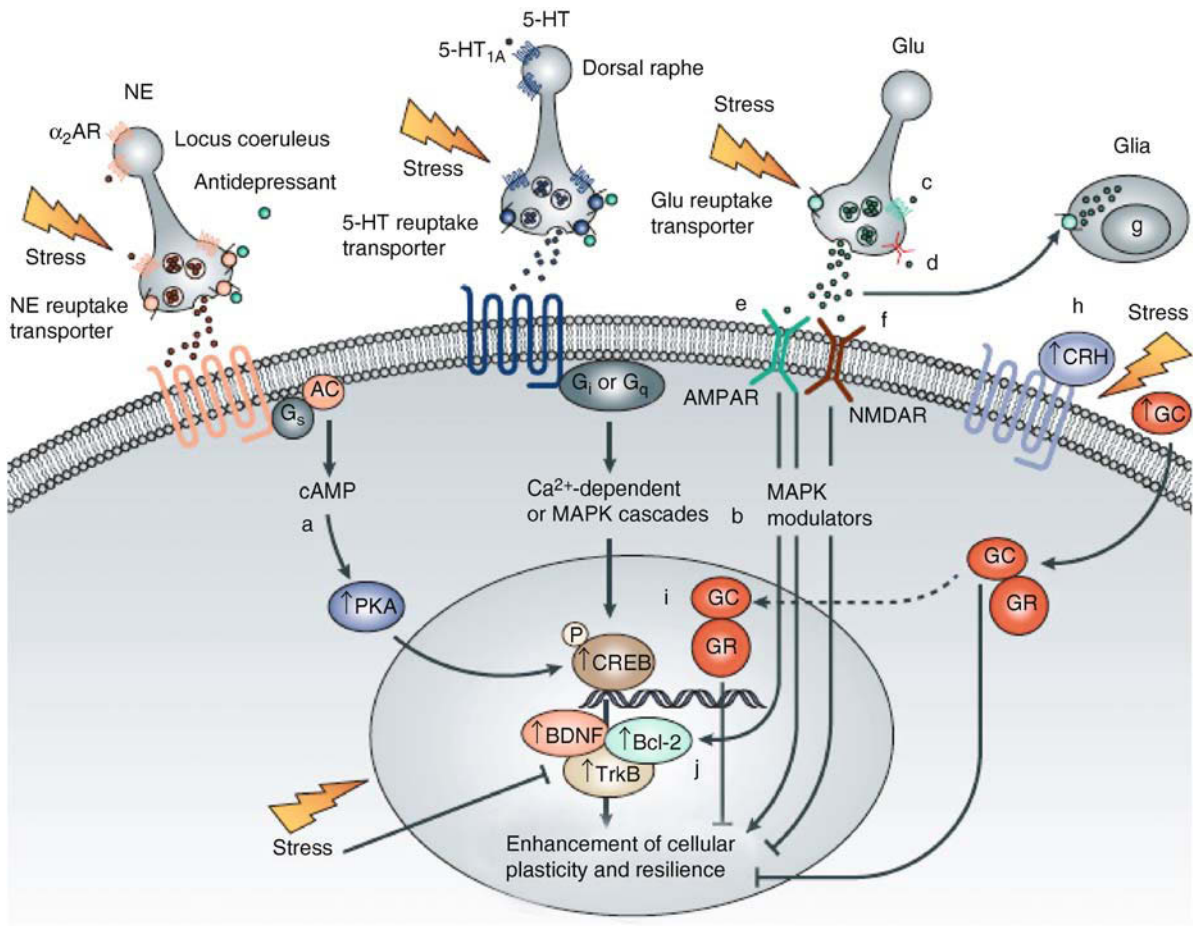
Antidepressants; Mood elevators; Psycho energizers

Definition

Antidepressants are small heterocyclic molecules entering the circulation after oral administration and passing the blood–brain barrier to bind at numerous specific sites in the brain. They are used for treatment of depression, panic disorders, generalized anxiety disorder, social phobia, obsessive compulsive disorder, and other psychiatric disorders and nonpsychiatric states.

Mechanism of Action

Most available antidepressants enhance neurotransmission of ►biogenic amines, mainly ►norepinephrine and ►serotonin, to a lesser extent dopamine. Once released from specialized vesicles at the presynaptic nerve terminal ►neurotransmitters enter the synaptic cleft and bind to respective receptors at the postsynaptic cell membrane, thus modulating the associated signaling cascades (Fig. 1). Additionally, some of them bind to presynaptically localized receptors that regulate the amount of transmitter released. The cell membrane of presynaptic nerve terminals also contains ►reuptake transporters that clear the synaptic cleft from biogenic amines. Once reshuffled into the presynaptic compartment the neurotransmitter is degraded by ►monoamine oxidase (MAO). These two molecular processes, reuptake through specific transporters and enzymatic degradation by MAO, are targeted by most of the antidepressant drugs. For example, the ►selective serotonin reuptake inhibitors (SSRI; citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline) and the so called “dual acting drugs” (the selective serotonin/noradrenaline reuptake inhibitors venlafaxine and duloxetine and the noradrenergic/selective serotonergic drug mirtazapine), which became the mainstay for the treatment of the majority of depressed patients either prevent clearance of serotonin and/or noradrenaline from the synaptic cleft by blocking the presynaptic transporter and thus amplify receptor-mediated events postsynaptically or antagonize specific pre- and postsynaptic receptors. Analogous effects are those by norepinephrine reuptake inhibitors, while MAO-inhibitors act by reducing norepinephrine or serotonin degradation and thus increase the releasable amount of



Antidepressant Drugs. Figure 1 Effects of stress as a model for depression and the reversal by the use of antidepressants. Multiple intracellular targets might be involved in the regulation of plasticity and resilience by antidepressants, which block extracellular transporters. Adapted from [1].

neurotransmitter from the respective vesicles. These drugs do not exert prompt antidepressant effects as it takes weeks or months until clinical amelioration occurs. The exact mode of action by which antidepressants work is still not resolved but there is consensus that their primary action, i.e., binding to cell membrane transporters, triggers a manifold of events, which we are only beginning to decipher (Fig. 1; [1]). Ingredients of St. John's wort have serotonin-/noradrenaline-/GABA- and glutamate reuptake inhibiting properties. The older tricyclic antidepressants like amitriptyline are also noradrenaline and/or serotonin reuptake inhibitors but also show antagonistic effects on different cerebral receptors like α 1-adrenergic, cholinergic, histaminergic receptors.

One such hypotheses submits that most antidepressants enhance the expression of cyclic-AMP response element binding protein (CREB), which is a transcription factor that after phosphorylation binds to cyclic-AMP response elements localized in the promoter region of many genes including that coding for ►brain

derived neurotrophic factor (BDNF) [2]. The latter neurotrophin was found to be decreased in the hippocampus of chronically stressed rats, serving as animal model of depression. When treated with antidepressants, BDNF expression increases, possibly through enhanced phospho-CREB driven transactivation of the *BDNF* gene. This hypothesis is in keeping with the frequently observed reduction of depressed patients hippocampus volume (estimated by magnetic resonance imaging), a limbic brain structure pertinent for cognitive function, and expressing BDNF at high levels. Some preliminary studies support that antidepressants increase adult neurogenesis in this brain area, a phenomenon also associated with increased levels of phospho-CREB. The hypothesis that phospho-CREB is involved in adult neurogenesis is also strengthened by experiments with transgenic mice overexpressing a dominant negative isoform of CREB where the Ser¹³³ is mutated preventing phosphorylation-induced transactivation of CREB. Overexpression of mutant CREB prevented decreased neurogenesis in adult

hippocampus. While many pieces of this hypothesis are in line with an antidepressant-induced enhancement of neurogenesis, evidence is lacking that this effect is the same through which antidepressants regulate emotional states. Morphological studies on brains of depressives failed to detect evidence for neuronal deterioration in the hippocampus. Moreover the increase of *BDNF* gene transcription as induced by antidepressants is possibly an unspecific response to a xenobiotic molecule. Whether increased transcription of *BDNF* conveys antidepressant effects is yet not proven, as mouse mutants where *BDNF* production is lowered by heterozygous gene deletion failed to show behavioral abnormalities. Also, data on drug-induced changes in *BDNF* peptide concentrations are not giving a clear picture.

Another hypothesis derives from the clinical observation that impaired stress hormone regulation is a cardinal symptom among patients with an acute major depressive episode. If stress hormones (primarily cortisol secreted by adrenocortical glands and corticotropin released from the pituitary) are monitored longitudinally in these patients, those who respond to drug treatment show a trend towards neuroendocrine normalization while those where stress hormone regulation continues to be altered have a much worse outcome, i.e., they fail to respond or they relapse. Studies using transgenic mice with glucocorticoid receptor impairment show some behavioral and functional features reminiscent of depression. Some of these abnormalities disappear under antidepressants, which is in line with a drug-induced improvement of corticosteroid receptor function. When ligand-activated, these glucocorticoid and mineralocorticosteroid receptors form homo- and heterodimers that interact with **corticotropin releasing hormone** (CRH) in many ways (Fig. 2). This is of relevance in this context because clinical and basic studies have shown that overexpression of CRH in many brain areas is causally related to development and course of depression. The effect of antidepressants has therefore consequences upon CRH secretion and it is believed that these antidepressants may work through this corticosteroid receptor driven signaling pathway, suppressing the depressogenic and anxiogenic effects of CRH acting through CRH type 1 receptors (CRHR1). Thus, the antidepressant-induced behavioral and neuroendocrine changes in patients together with their observed molecular actions upon stress hormone signaling pathways have triggered the search for new pharmacological approaches to understand how antidepressants might work and ultimately to discover better drugs.

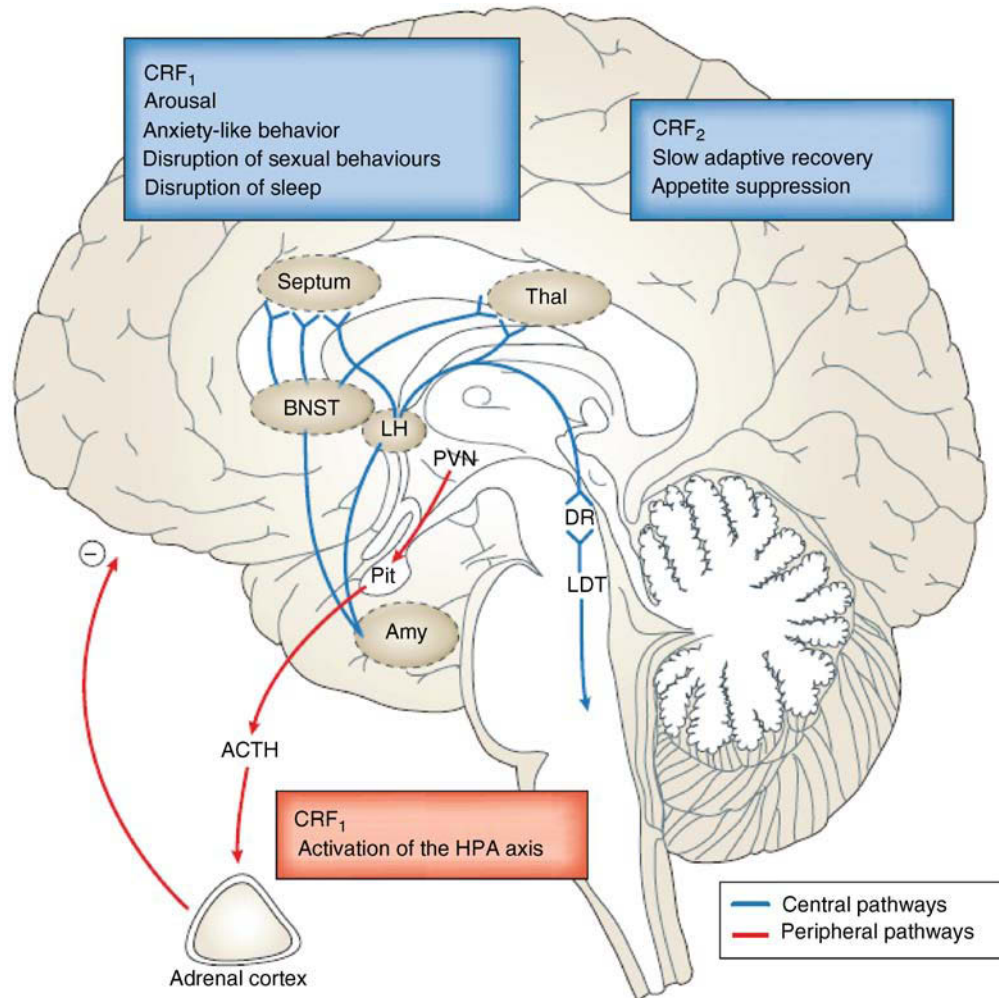
In the absence of a robust pathogenetic model for depression, hypotheses-driven research has limitations that hopefully can be overcome by unbiased approaches. The availability of cDNA microarrays allowing

one to study a huge amount of genes, which are simultaneously regulated in the brains of mice under long-term treatment with antidepressants will shift the emphasis from the “usual suspects” (such as serotonin and its receptors) to yet unheard candidate genes [3].

Clinical Use and Side Effects

Antidepressants were serendipitously discovered in the 1950s and the first generation of these drugs was constituted by tricyclic molecules. The refinement among the second and third generation of these drugs resulted in molecules that have less side effects, are better tolerated and consequently enjoy much better acceptance. In fact, the percentage of Americans treated for depression tripled nationwide. Simultaneously patient visits to doctors for depression fell by a third through the last 5 years. Such figures do not yet apply for Europe, where alternative treatments, especially herbals (St. Johns Wort is the best selling antidepressant in Germany), continue to play a major role. Given the personal and socioeconomic burden of depression the under-treatment of this disabling clinical condition seems neither ethical nor prudent.

While antidepressants have proven to be effective drugs, several drawbacks and caveats need to be resolved. This can be most likely achieved by enforced **pharmacogenetic approaches** (**pharmacogenetics**) in combination with refined clinical research: Matching patients to the antidepressant that is most likely to be effective and less likely to harm through adverse reactions is the main goal of all modern therapies. Patient characteristics including sex, age, anxiety level, premedication, and family history (genetic load) do not predict better or worse response to a particular antidepressant drug or drug class. However, the fact that all drugs are equally effective between comparison groups does not mean that they are equally effective for individual patients. It is now hoped that combination of clinical data, including functional assessments, e.g., neuroendocrine, neuroimaging, neuropsychology together with information from genotyping, i.e., identification of a collection of single nucleotide polymorphisms (SNPs) will ultimately lead to choosing a first-line antidepressant based upon individual data. The most frequently examined candidate gene codes for the serotonin transporter. A 44-bp insertion or deletion results in a long and a short variant of this gene; the s-variant is associated with a twofold decreased expression and transport activity in vitro. Individuals in an epidemiological sample with one or two copies of the short allele of the serotonin transporter promoter polymorphism showed more depressive symptoms, diagnosable depression, and tendency to commit suicide in relation to stressful life events than individual homozygous for the long allele.



Antidepressant Drugs. Figure 2 The corticotropin-releasing factor system in depression. Corticotropin releasing factor (CRF) from the paraventricular nucleus (PVN) of the hypothalamus is released into the hypophyseal portal system and triggers the release of corticotrophin (ACTH) from the anterior pituitary via stimulation of CRF₁ receptors. ACTH, in turn, stimulates the secretion of glucocorticoid hormones (cortisol in humans or corticosterone in rodents) from the adrenal cortex. Increased glucocorticoid levels suppress hypothalamic CRF expression via negative feedback through hippocampal and hypothalamic glucocorticoid receptors. The neurotransmitter action of CRF on CRF₁ receptors throughout the limbic system mediates anxiogenic effects of stress. By contrast, its neurotransmitter action on CRF₂ receptors in more discrete regions of the brain might reduce anxiety-like behavior in a delayed fashion (adapted from [2]).

Unfortunately, twin studies were not concordant in lending support to this result [4].

In practice, a genotype-guided medication selection is yet not in reach, but several minor innovations emerging from hypothesis-driven research are. The current antidepressive pipeline contains three promising candidates: ► **Substance P**, a peptide from the tachykinin family, which binds preferentially at the NK₁-receptor, was suspected to play a role in causality of depression. Several clinical studies testing NK₁-receptor antagonists showed promising results and a number of pharmaceutical companies are developing

drugs antagonizing NK- receptors. NK₂-receptor antagonists might also be effective in the treatment of depression. Another neuropeptide is CRH that seems to be causally related to symptoms of depression through activation of CRHR1, which led to the development of CRHR1 antagonists as potential antidepressants. A first clinical study supported such a possibility. Finally, glutamate antagonists like ketamine might have some use in treatment-resistant depression.

Another new development of immediate clinical usefulness is the analysis of genetic variability in the cytochrome P450 enzyme system in patients, which

may elucidate clinically relevant changes in drug metabolism and adverse reactions. For example, if a patient receives an SSRI such as Prozac, which blocks the P4502D6 enzyme, and an antiarrhythmic, which is metabolized by the same enzyme, a fatal increase of the cardiotoxic drug may occur. Other possible candidates involved in pharmacokinetics are P-glycoproteins, which are important regulators of a drug's blood-brain barrier passage. It was recently shown that antidepressants are substrates of P-glycoprotein, which, if over-expressed, can extrude the antidepressant out of the brain cells into the circulation thus preventing central effects that may lead to therapy resistance.

Side effects of antidepressants usually occur during the first days of treatment and tend to diminish over time. The side-effect profile can be easily derived from the transporter-binding profile. Serotonergic drugs might cause headaches, appetite loss, nervousness, sweating and sexual dysfunction and noradrenergic drugs palpitations, sweating, anxiety, and drowsiness. Anticholinergic antidepressants show side effects like constipation, blurred vision, memory dysfunction, dry mouth, while antihistaminergic drugs exhibit side effects like sedation, hypotension, and weight gain. Antiadrenergic properties are associated with postural hypotension and reflex tachycardia.

- ▶ Neurotransmitter Transporters
- ▶ Noradrenaline Transporter
- ▶ Serotonergic System
- ▶ Monoamine Oxidases

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Antidiabetic Drugs

Antidiabetic drugs is the general term for drugs that lower blood glucose concentrations and are used in the treatment of diabetes mellitus. Antidiabetic drugs are

typically categorized as either oral (sulphonylureas, prandial insulin releasers, metformin, thiazolidinediones, alpha-glucosidase inhibitors) which are used to treat most type 2 (non-insulin-dependent) diabetic patients, or insulin (given parenterally) which is used to treat all type 1 and some type 2 diabetic patients.

- ▶ Diabetes Mellitus
- ▶ Insulin
- ▶ Antidiabetic Drugs other than Insulin

Antidiabetic Drugs other than Insulin

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Synonyms

Oral hypoglycaemic agents; Oral blood glucose-lowering drugs; Insulin secretagogues; Antihyperglycaemics

Definition

Antidiabetic drugs are used to treat hyperglycaemia in type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus. They are used in conjunction with non-pharmacological interventions involving diet, exercise and health education. Insulin therapy is essential for all type 1 patients and is often used to treat more advanced stages of type 2 diabetes. Other antidiabetic drugs are mostly used to treat type 2 diabetes, which accounts for more than 85% of all cases of diabetes. The classes of antidiabetic drugs other than insulin are sulphonylureas, prandial insulin releasers (also termed meglitinides), the biguanide metformin, thiazolidinediones (TZDs), α -glucosidase inhibitors, incretin mimetics, gliptins (also termed dipeptidyl peptidase-4 inhibitors or incretin enhancers) and the amylin analogue pramlintide (Table 1) [1].

Mechanism of Action

Type 2 diabetes is a heterogeneous and progressive endocrine disorder associated with insulin resistance (impaired insulin action) and defective function of the insulin-secreting β -cells in the pancreatic islets of Langerhans. These endocrine disorders give rise to widespread metabolic disturbances epitomised by hyperglycaemia. The present classes of antidiabetic agents other than insulin act to either increase insulin secretion, improve insulin action, slow the rate of intestinal

Antidiabetic Drugs other than Insulin. Table 1 Classes of antidiabetic drugs other than insulin and their main mechanisms of action

Class	Examples ^a	Main mechanism of action	Route
Sulphonylureas	Chlorpropamide, glibenclamide ^b , gliclazide, glimepiride, glipizide, gliquidone, tolazamide, tolbutamide	Stimulate insulin secretion (typically 6–24 h)	Oral
Prandial insulin releasers (meglitinides)	Repaglinide, nateglinide	Stimulate insulin secretion (rapid and short-acting < 6 h)	Oral
Biguanide	Metformin	Improve insulin action	Oral
Thiazolidinediones	Pioglitazone, rosiglitazone	Improve insulin action (PPAR γ agonists)	Oral
α -Glucosidase inhibitors	Acarbose, miglitol, voglibose	Slow rate of carbohydrate digestion	Oral
Incretin mimetic	Exenatide	Mimic GLP-1 ^c : enhance prandial insulin secretion	SC injection ^d
Gliptins (DPP-4 inhibitors)	Sitagliptin	Inhibit DPP-4 ^e : enhance prandial insulin secretion	Oral
Amylin analogue	Pramlintide	Suppress glucagon secretion and slow gastric emptying	SC injection ^c

^aAvailability of agents and prescribing instructions vary between countries.

^bGlibenclamide is called glyburide in some countries.

^cGlucagon-like peptide-1.

^dSubcutaneous injection.

^eDipeptidyl peptidase-4.

carbohydrate digestion, enhance the incretin effect, suppress glucagon secretion or slow gastric emptying.

Sulphonylureas

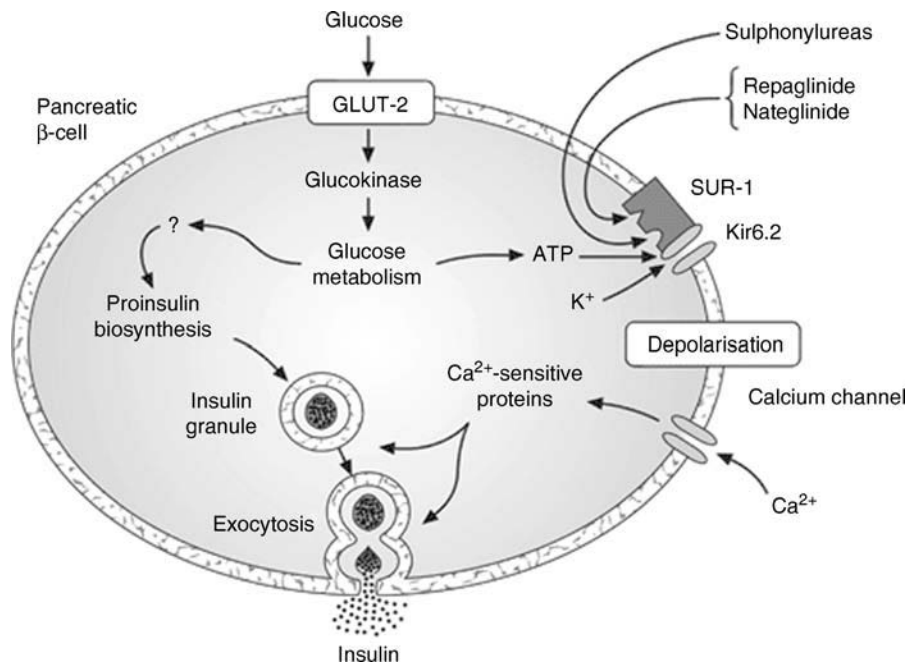
The first sulphonylureas were introduced in the 1950s. They stimulate insulin secretion by a direct effect on pancreatic β -cells. Sulphonylureas enter the β -cell and bind to a site at the cytosolic face of the sulphonylurea receptor (SUR). The SUR-1 isoform is expressed by the β -cell. It forms part of a transmembranal complex that includes ATP-sensitive Kir6.2 potassium efflux channels (K-ATP channels). The binding of a sulphonylurea to SUR-1 produces a conformational change that closes K-ATP channels, favouring local depolarisation of the plasma membrane. This opens voltage-dependent L-type calcium channels, increasing calcium influx and raising the cytosolic free calcium concentration. In turn, this activates calcium-dependent signalling proteins controlling the contractile activities of microtubules and microfilaments that mediate exocytosis of insulin granules. Preformed insulin granules adjacent to the plasma membrane are released first (first-phase insulin release). Newly formed granules contribute to the secretory pool within 1 h of continued stimulation. Increased insulin release is sustained as long as drug stimulation is maintained, provided the β -cells are functionally competent (Fig. 1).

The SUR–Kir6.2 complex is a non-covalently bonded octamer ($4 \times \text{SUR}/4 \times \text{Kir6.2}$), with the pore-forming Kir6.2 channels located at the centre (Fig. 2).

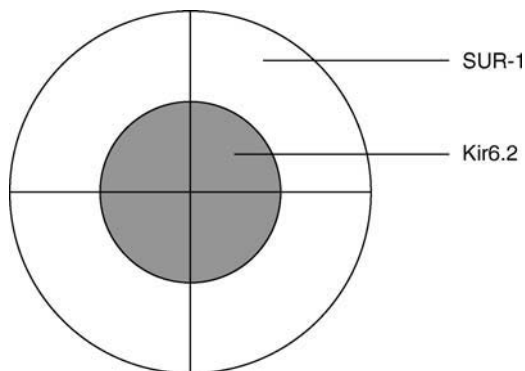
SUR molecules are members of the ATP binding cassette proteins (ABC proteins). Each SUR-1 molecule comprises 17 transmembrane domains, 2 cytosolic nucleotide binding domains and cytosolic binding domains for sulphonylurea, benzamido and other ligands. The Kir6.2 channel also has cytosolic binding regions, including one for ADP/ATP. Sulphonylureas bind to the sulphonylurea site with high affinity (e.g. K_i for glibenclamide in low nanomolar range), being dependent on a ‘U’ shape to the ligand with 5.5 Å between the hydrophobic rings.

By closing K-ATP channels, sulphonylureas induce insulin release by activating a step along the normal pathway of glucose-induced insulin secretion. Activation of insulin secretion is therefore independent of glucose, provided there is sufficient glucose metabolism to stimulate proinsulin biosynthesis and service the energy requirements for the cellular processing and exocytosis of insulin. Hence sulphonylureas can stimulate insulin secretion at low glucose concentrations, creating the risk of hypoglycaemia. Sulphonylureas will also increase the amount of insulin secreted at any level of stimulation by glucose, subject to adequate β -cell function. Additionally, sulphonylureas may potentiate insulin release that is stimulated by glucose and other nutrients. This may involve SUR molecules located within the membranes of insulin granules and activation of certain isoforms of protein kinase C.

Although the main therapeutic effect of sulphonylureas is increased insulin secretion, there is evidence that



Antidiabetic Drugs other than Insulin. Figure 1 Sulphonylureas stimulate insulin release by pancreatic β-cells. They bind to the sulphonylurea receptor (SUR-1), which closes Kir6.2 (ATP-sensitive) potassium channels. This promotes depolarisation, voltage-dependent calcium influx, and activation of calcium-sensitive proteins that control exocytotic release of insulin.



Antidiabetic Drugs other than Insulin. Figure 2 Octameric structure (4 × SUR/4 × Kir6.2) of the SUR–Kir6.2 complex.

these drugs exert weak extra-pancreatic effects. The latter effects include suppression of hepatic gluconeogenesis, possibly by suppression of a kinase which leads to increased formation of fructose-2, 6-bisphosphate. This stimulates phosphofructokinase and suppresses fructose-1, 6-bisphosphatase, thereby increasing glycolytic flux and suppressing gluconeogenic flux. Sulphonylureas might also enhance insulin-stimulated glucose transport by increasing translocation of GLUT-4 glucose transporters to the plasma membrane in adipocytes and muscle. However, these effects appear to require

supra-therapeutic concentrations of sulphonylureas and are probably not therapeutically relevant. Sulphonylureas have been reported to reduce the hepatic extraction of insulin and to act on pancreatic α-cells to transiently stimulate and then suppress glucagon secretion.

The increase in insulin concentrations produced by sulphonylureas lowers blood glucose concentrations through decreased hepatic glucose output and increased glucose utilisation, mostly by muscle (► [insulin](#), ► [insulin receptor](#)).

Prandial Insulin Releasers (Meglitinides)

This class comprises the meglitinide analogue repaglinide (introduced in 1998) and the structurally related D-phenylalanine analogue nateglinide (introduced in 2001). These agents have a benzamido group that binds to a site on SUR-1 that is distinct from the sulphonylurea site, but probably in close proximity and capable of binding interference. Some sulphonylureas also have a benzamido moiety (e.g. glibenclamide, glimepiride, glipizide) but the binding affinity for the sulphonylurea site has a higher affinity. Binding of repaglinide or nateglinide to the benzamido site closes the K-ATP channels and induces insulin secretion via the same pathway described for sulphonylureas.

Repaglinide and nateglinide are rapidly absorbed; their binding durations to SUR-1 are much shorter than sulphonylurea binding, and their hepatic metabolism

and subsequent elimination are faster. Consequently, repaglinide and nateglinide are faster-acting and shorter-acting insulin releasers than sulphonylureas. They can be taken immediately before a meal, and quickly stimulate insulin secretion to coincide approximately with the period of meal digestion, hence the categorisation of 'prandial insulin releasers'.

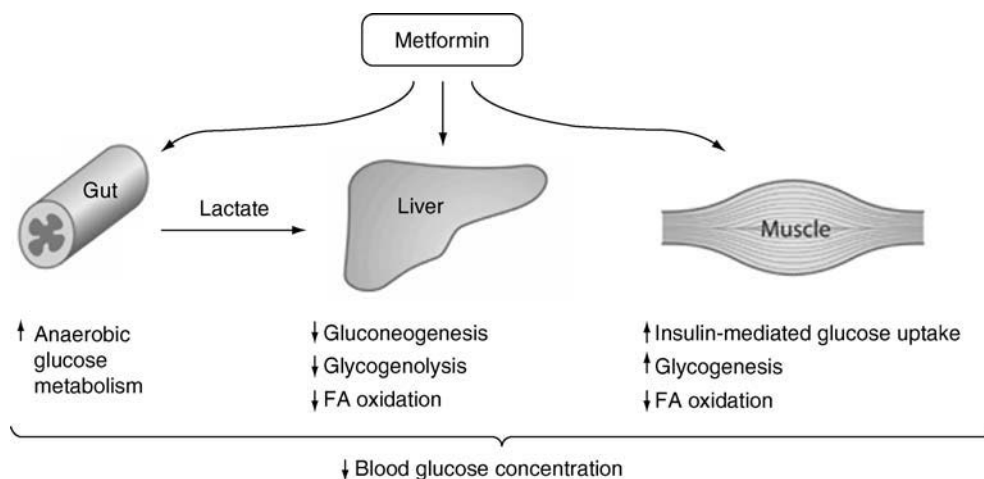
Biguanide

Metformin is the main compound in this class, introduced in the late 1950s. Other biguanides, namely phenformin and buformin have been widely discontinued. The antihyperglycaemic effect of metformin results partly from a direct improvement of insulin action and partly from actions that are not directly insulin dependent. A presence of insulin is required for the therapeutic efficacy of metformin, but the drug does not stimulate insulin release and is often associated with a small decrease in basal insulin concentrations in hyperinsulinaemic patients. Metformin has a variety of metabolic effects: The main antihyperglycaemic actions involve a reduction of excess hepatic glucose production, increased insulin-mediated glucose utilisation predominantly by muscle, decreased fatty acid oxidation and increased splanchnic glucose turnover.

Metformin restrains hepatic glucose production principally by suppression of gluconeogenesis. The mechanisms involve potentiation of insulin action and decreased hepatic extraction of certain gluconeogenic substrates such as lactate. In addition, metformin reduces the rate of hepatic glycogenolysis and decreases the activity of hepatic glucose-6-phosphatase. Insulin-stimulated glucose uptake and glycogenesis by skeletal muscle is increased by metformin mainly by increased

movement of insulin-sensitive glucose transporters (GLUT-4) into the plasma membrane. Metformin also appears to increase the transport function of glucose transporters and increases the activity of glycogen synthase. Further actions of metformin include insulin-independent suppression of fatty acid oxidation in liver and muscle, and insulin-independent increase in anaerobic glucose metabolism by the intestine. Lactate produced in this way is recycled to glucose by the liver. Thus metformin acts to a modest extent via several different effects to lower blood glucose concentrations (Fig. 3).

Metformin enters some cell types (e.g. liver) at least in part via the organic cation transporter 1. The drug improves insulin sensitivity by increasing insulin-stimulated tyrosine kinase activity of the β -subunit of the insulin receptor, possibly by reducing phosphatase-mediated receptor dephosphorylation. Metformin also increases insulin signalling at more distal steps in the postreceptor cascades. Although metformin can increase insulin receptor binding when insulin receptor numbers are depleted, this does not appear to have a significant impact on insulin action. The mediating steps that enable metformin to interface with insulin-signalling pathways are not resolved. Metformin has been shown to alter membrane fluidity in hyperglycaemic states and to alter the activities of several metabolic enzymes (listed above), apparently independently of insulin. Emerging evidence suggests that metformin can activate adenosine monophosphate-activated protein kinase (AMPK) via an LKB1-dependent mechanism. Very high concentrations of metformin that occur in the intestine could increase anaerobic glucose metabolism by suppression of the respiratory chain at complex I.



Antidiabetic Drugs other than Insulin. Figure 3 The antihyperglycaemic effect of metformin involves enhanced insulin-mediated suppression of hepatic glucose production and muscle glucose uptake. Metformin also exerts non-insulin-dependent effects on these tissues, including reduced fatty acid oxidation and increased anaerobic glucose metabolism by the intestine. FA, fatty acid; ↑, increase; ↓, decrease.

Thiazolidinediones

Two TZDs introduced in 1999 are presently available, pioglitazone and rosiglitazone. Another TZD, troglitazone has been withdrawn. TZDs improve insulin sensitivity and their principal mechanism of action is stimulation of the nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR γ). PPAR γ (►PPARs) is a member of the nuclear receptor superfamily for retinoid, steroid and thyroid hormones. PPAR γ exists as a heterodimer with the retinoid X receptor (RXR). Binding of a TZD to PPAR γ together with binding of *cis*-retinoic acid to the RXR moiety produces a conformational change that prompts dissociation of co-repressors. The activated heterodimer then binds to the peroxisome proliferator response element (PPRE), which is a sequence (AGGTCAAGGTCA) located in the promoter region of the responsive genes. Recruitment of co-activators including PGC-1 and assembly of the RNA polymerase complex follows, initiating transcription (Fig. 4). Many of the responsive genes are also activated by insulin, hence the ability of TZDs to improve insulin sensitivity [2].

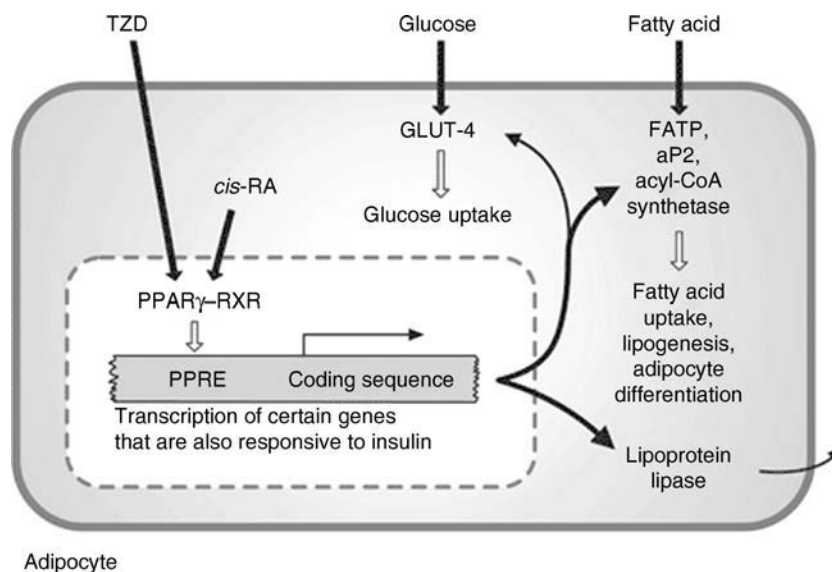
PPAR γ is strongly expressed in adipocytes, and stimulation by TZDs promotes adipogenesis, predominantly in preadipocytes from subcutaneous depots. Increased transcription of transporters and enzymes involved in fatty acid uptake and lipogenesis increases the deposition of lipid in these adipocytes (Table 2). This appears to facilitate a reduction in hyperglycaemia by reducing circulating concentrations of non-esterified

(free) fatty acids and triglycerides. The consequent effect on the glucose-fatty acid (Randle) cycle is to reduce the availability of fatty acids as an energy source, thereby favouring the utilisation of glucose. Additionally, TZDs increase transcription of GLUT-4 glucose transporters that directly facilitates glucose uptake. Reducing free fatty acid concentrations also reduces the production of lipid metabolites, which suppress early postreceptor steps in the insulin-signalling pathway. TZDs may further improve insulin signalling by increasing production of the adipocyte hormone adiponectin, decreasing production of the adipocyte cytokine tumour necrosis factor- α (TNF α), and decreasing production of the adipocyte hormone resistin (and possibly leptin), which have been implicated in the pathogenesis of insulin resistance.

There is weak expression of PPAR γ in muscle, liver and other tissues, enabling TZDs to support the effects of insulin in these tissues, notably increased glucose uptake in muscle and reduced glucose production in liver. TZDs may also affect nutrient metabolism by skeletal muscle through a direct mitochondrial action that is independent of PPAR γ .

α -Glucosidase Inhibitors

The first member of this class, acarbose, was introduced in the early 1990s. α -Glucosidase inhibitors slow the intestinal process of carbohydrate digestion by competitive inhibition of the activity of α -glucosidase enzymes located in the brush border of the enterocytes



Antidiabetic Drugs other than Insulin. Figure 4 Thiazolidinediones stimulate the PPAR γ moiety of the PPAR γ RXR nuclear receptor complex, which then binds to a response element, leading to transcription of certain genes that are also responsive to insulin. These facilitate increased uptake of fatty acids, lipogenesis and adipogenesis. PPAR γ , peroxisome proliferator-activated receptor- γ ; RXR, retinoid X receptor; PPRE, peroxisome proliferator response element; TZD, thiazolidinedione; *cis*-RA, *cis*-retinoic acid; GLUT-4, glucose transporter isoform-4; FATP, fatty acid transporter protein; aP2, adipocyte fatty acid binding protein.

(Fig. 5). Acarbose also causes a modest inhibition of pancreatic α -amylase activity. The principal α -glucosidase enzymes are glucoamylase, sucrase, maltase and dextrinase. The inhibitors bind to these enzymes with much higher affinity than their natural disaccharide and oligosaccharide substrates. Hence, when bound to the inhibitor, the enzyme fails to cleave the disaccharides and oligosaccharides into their absorbable monosaccharides. The available α -glucosidase inhibitors, acarbose, miglitol and voglibose, show different binding affinities for the enzymes, giving them different activity profiles. For example, the affinity profile of acarbose is glycoamylase > sucrase > maltase > dextrinase. Miglitol is a more potent inhibitor of sucrase, and voglibose of other α -glucosidases [3].

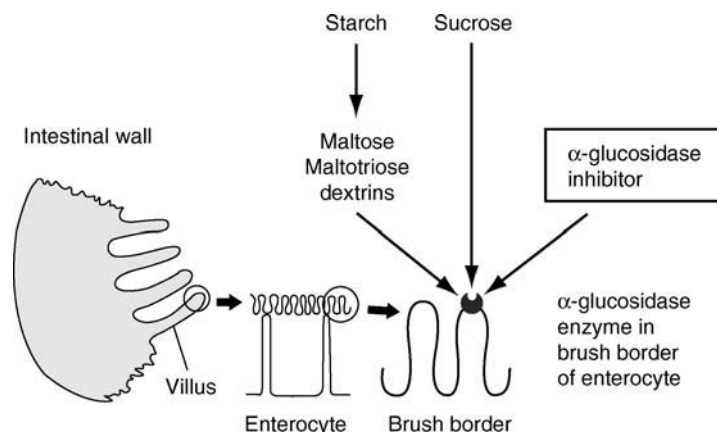
When α -glucosidase activity is inhibited, carbohydrate digestion is prolonged and takes place further along the intestinal tract. This in turn delays and spreads the period of glucose absorption, which reduces the extent of the postprandial rise in blood glucose concentrations. The effectiveness of α -glucosidase inhibitors is dependent on the consumption of a meal rich in complex carbohydrate.

Incretin Mimetics

The first incretin mimetic 'exenatide' was introduced in 2005. It is an analogue of the gut hormone glucagon-like peptide-1 (GLP-1). This therapy is based on the so-called 'incretin' effect, which is the enhanced insulin response to nutrients that occurs after a meal (compared with the insulin response to similar plasma nutrient levels created by intravenous administration). The incretin effect is due to hormonal and neural stimuli produced by the gut during meal digestion which increase glucose-induced insulin secretion, and thereby reduce prandial glucose excursions. The main incretin hormones are GIP (glucose-dependent insulintropic polypeptide), produced by K-cells in the mucosa of the duodenum and jejunum, and GLP-1 from L-cells located mostly in the mucosa of the ileum. Both GIP and GLP-1 increase glucose-stimulated insulin secretion. Additionally, GLP-1 reduces glucagon secretion from pancreatic α -cells in a glucose-dependent manner, slows gastric emptying and exerts a satiety effect (Table 3). Animal and in vitro studies have suggested that GIP and GLP-1 might increase neogenesis and proliferation of β -cells and reduce β -cell apoptosis, but

Antidiabetic Drugs other than Insulin. Table 2 Tissue expression, ligands, genes activated, and biological actions of the peroxisome proliferator-activated receptor- γ (PPAR γ)

Tissue expression	Mainly white and brown adipose tissue; weak expression in liver, muscle, gut, macrophages, pancreatic β -cells and haemopoietic tissues
Natural ligands	Certain unsaturated fatty acids and prostaglandin metabolites
Synthetic ligands	Thiazolidinediones and some non-steroidal antiinflammatory drugs
Gene activated	Lipoprotein lipase; fatty acid transporter protein; adipocyte fatty acid binding protein; acyl-CoA synthetase; malic enzyme; GLUT-4 glucose transporter; phosphoenolpyruvate carboxykinase
Biological actions	Adipocyte differentiation; fatty acid uptake; lipogenesis; glucose uptake; other effects on nutrient metabolism which lower hepatic glucose production



Antidiabetic Drugs other than Insulin. Figure 5 α -Glucosidase inhibitors slow the rate of intestinal carbohydrate digestion by competitive inhibition of α -glucosidase enzymes in the brush border of enterocytes. The α -glucosidase inhibitors have a higher affinity for the α -glucosidase enzymes than the natural disaccharide and oligosaccharide substrates.

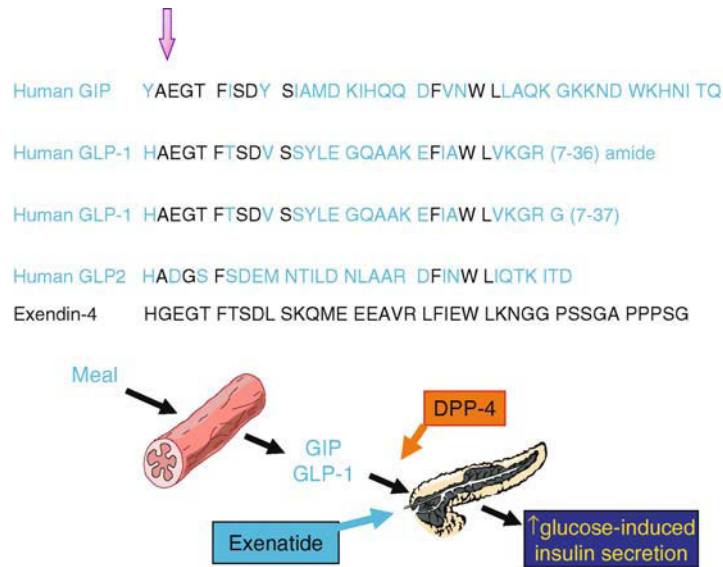
it is uncertain whether these hormones can preserve β -cell mass in human type 2 diabetes [4].

The incretin effect is reduced in type 2 diabetes, and this is attributed, at least in part, to reduced secretion of GLP-1. The biological actions of GLP-1 remain essentially intact in type 2 diabetes, but administration of extra GLP-1 is not a practical therapeutic option because the peptide is degraded rapidly ($t_{1/2} < 2$ min) by the enzyme dipeptidyl peptidase IV (DPP-4). DPP-4 cleaves the N-terminal dipeptide from many of the peptides that have either an alanine or a proline residue penultimate to the N-terminus (Fig. 6).

Exenatide (exendin-4) is a GLP-1 analogue with a 52% sequence homology in which the penultimate N-terminal alanine residue of GLP-1 is replaced by glycine. This confers resistance to degradation by DPP-4, giving exenatide protracted biological activity of about 5–7 h after subcutaneous injection. Since exenatide interacts with the same receptor as GLP-1 and retains the same profile of biological effects as GLP-1, exenatide enables the incretin effect to be enhanced. It is noteworthy that DPP-4 exists free in the circulation and tethered to the external surface of endothelia and other epithelial cells in most tissues: it is also the CD26

Antidiabetic Drugs other than Insulin. Table 3 Actions of the incretin hormones GIP (glucose-dependent insulinotropic polypeptide, gastric inhibitory peptide) and GLP-1 (glucagon-like peptide-1)

	GIP	GLP-1
Pancreatic		
↑ Glucose-induced insulin secretion	Yes	Yes
↑ Proinsulin biosynthesis	Yes	Yes
↑ β -Cell survival (rodents)	Yes	Yes
↓ Glucagon secretion	–	Yes
Other actions		
↓ Gastric emptying	No (<i>slight</i>)	Yes
↓ Appetite/feeding	No	Yes
↓ Weight gain	No	Yes
↑ Myocardial glucose metabolism	–	Yes?
Type 2 Diabetes	Incretin effect reduced	
Postprandial response	About normal	Reduced (<i>late phase</i>)
Insulin-releasing effectiveness	Reduced	Retained (<i>mostly</i>)



Antidiabetic Drugs other than Insulin. Figure 6 The incretin mimetic, exenatide is a long-acting analogue of the gut hormone GLP-1. Arrow indicates the site of action of dipeptidyl peptidase-IV (DPP-4). Exenatide is resistant to degradation by DPP-4, allowing protracted GLP-1 mimetic activity that enhances the ‘incretin’ effect.

T-cell activating antigen although the aminopeptidase and immunological functions do not appear to interfere with each other.

Exenatide was discovered in the saliva of a lizard from Arizona – the Gila monster (*Heloderma suspectum*). For therapeutic purposes exenatide is usually administered by twice daily subcutaneous injection before the main meals. Because the insulin-releasing and glucagon-suppressing effects of exenatide (like GLP-1) are glucose dependent, there is low risk of severe hypoglycaemia. The satiety effect is often associated with some weight loss, and the slowing of gastric emptying can cause nausea, at least during initial therapy. About one third of patients develop antibodies to exenatide, but biological activity of the molecule is rarely affected.

Gliptins

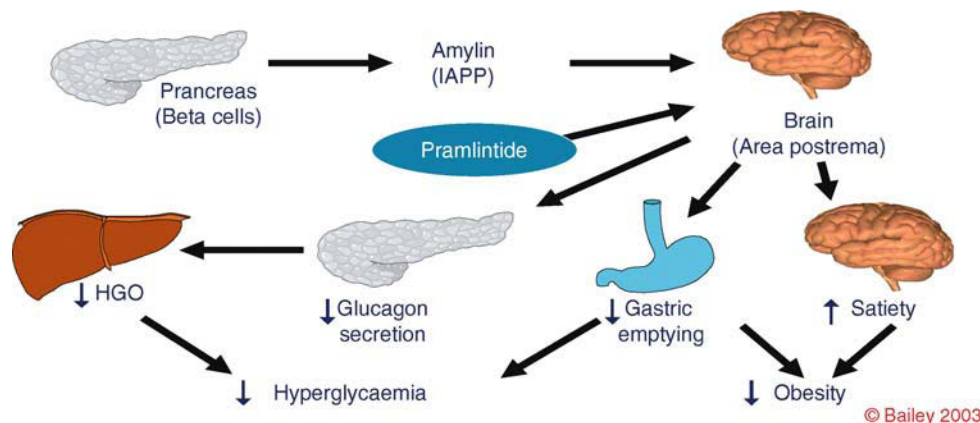
Gliptins (also termed dipeptidyl peptidase-IV inhibitors, DPP-4 inhibitors or incretin enhancers) are selective inhibitors of DPP-4 (described above). They enhance endogenous incretin activity by preventing the rapid degradation of GLP-1 and GIP (Fig. 6). The first gliptin ‘sitagliptin’ became available in the USA and UK in 2007. Since there are many other natural substrates for DPP-4 including neuropeptide Y (NPY), peptide YY (PYY), gastrin releasing polypeptide (GRP), substance P, insulin-like growth factor-1 (IGF-1), vasostatin-1 and several chemokines, gliptins have the potential to influence the hunger-satiety system, gastrointestinal motility, growth, vascular reactivity and immune mechanisms. However neither CD26 knockout mice nor the DPP-4-specific inhibitors used in animals or humans have yet shown any substantive untoward effects.

In clinical studies, selective DPP-4 inhibition increased active circulating concentrations of GLP-1 and GIP by two- to threefold. This was associated with increased glucose-induced insulin secretion and suppression of glucagon secretion, although changes in satiety and gastric emptying have not been reported.

Pramlintide

Pramlintide was introduced in the USA in 2005 as an adjunct to insulin therapy. It is a soluble analogue of the islet hormone amylin (islet amyloid polypeptide, IAPP) that is normally co-secreted from the pancreatic β -cells with insulin and C-peptide in response to nutrient stimuli. Paradoxically, amylin has been a suspect in the demise of β -cells in type 2 diabetes due to its accumulation and polymerisation to form insoluble fibrils in the islets. However normal amylin secretion appears to contribute to glucose homeostasis. Amylin acts centrally, probably via receptors in the area postrema (where there is no blood-brain barrier), dorsal raphe and nucleus accumbens. The central effects induce satiety and initiate a vagally-mediated suppression of prandial glucagon secretion and a slowing of gastric emptying (Fig. 7). In type 1 diabetes and advanced stages of type 2 diabetes there is a lack or substantial reduction of amylin. Thus replacement therapy with a non-aggregating analogue of amylin can be used to complement insulin therapy in type 1 and advanced type 2 diabetic patients.

The structure of pramlintide (Fig. 8) differs from human amylin by the substitution of three residues with proline residues, retaining biological potency but preventing self aggregation. Pramlintide is not used alone: it is administered by subcutaneous injection as an adjunct to insulin therapy. Since pramlintide requires a more acidic pH than insulin it has to be given as a separate injection to insulin, usually just before the main meals. The suppression of glucagon secretion and to a lesser extent the slowing of gastric emptying are the main immediate actions of pramlintide that reduce blood glucose. The satiety effect is typically associated with a long-term reduction of food intake and body weight: reduced adiposity in obese type 2 diabetes generally improves metabolic control. It is advised to reduce the mealtime insulin dose during initiation of pramlintide therapy to reduce the risk of interprandial hypoglycaemia. Antibodies to pramlintide have been



Antidiabetic Drugs other than Insulin. Figure 7 Mechanisms of action of the amylin analogue pramlintide.

identified in some patients although these do not appear to affect biological activity.

Clinical Use

Type 2 (non-insulin-dependent) diabetes typically emerges in middle or later life. Unlike type 1 diabetes in which there is total loss of pancreatic β -cells and a critical need for exogenous insulin administration, type 2 diabetes is associated with a continued presence of β -cells and continued insulin production. However insulin resistance usually develops as a prelude to type 2 diabetes and creates a demand for a compensatory increase in insulin secretion. Eventually, the β -cells are unable to produce sufficient extra insulin to overcome the insulin resistance. This results in impaired insulin-mediated glucose uptake by muscle, failure of insulin to suppress hepatic glucose production and consequently hyperglycaemia. Pancreatic β -cells of type 2 diabetic patients become increasingly sluggish in their responsiveness to raised glucose concentrations, and eventually β -cell function becomes severely impaired, leading to a state of hypoinsulinaemia and greater hyperglycaemia. The toxic effects of hyperglycaemia on the permeability of small blood vessels and

nerve function result in the long-term microvascular and neuropathic complications of diabetes (retinopathy, nephropathy and neuropathy). The additional effects of other metabolic disturbances associated with insulin resistance (the so-called 'metabolic syndrome') are largely responsible for the long-term cardiovascular complications of type 2 diabetes [5].

Achieving and maintaining blood glucose concentrations as close to normal as possible reduces the morbidity and premature mortality of the long-term complications of type 2 diabetes. All treatments begin with non-pharmacological measures (diet, exercise and healthy living), but compliance is limited, and lasting glycaemic control occurs in only a small minority of patients. Patients are usually started on one oral antidiabetic drug. Recent studies suggest that metformin offers additional advantages beyond glycaemic control to reduce long-term cardiovascular complications. Thus, this is often the first oral agent to be used. The mechanisms of action of metformin also prevent weight gain and avoid overswings into hypoglycaemia. Alternatively, a sulphonylurea or a prandial insulin releaser may be favoured as the first oral antidiabetic agent if substantial β -cell failure is suspected. The

Amylin	KCNTA TCATQ RLANF LVHSS NNFGA ILSST NVGSNT
Pramlintide	KCNTA TCATQ RLANF LVHSS NNFGP ILPPT NVGSNTY

Antidiabetic Drugs other than Insulin. Figure 8 Structure of human amylin and its soluble analogue pramlintide.

Antidiabetic Drugs other than Insulin. Table 4 Main exclusions, adverse events and precautionary monitoring required for clinical use of oral anti-diabetic drugs

Class ^a	Main exclusions	Main adverse events	Monitoring ^b
Sulphonylureas	Severe liver or renal disease ^c	Hypoglycaemia	— ^b
Prandial insulin releasers (meglitinides)	Severe liver or renal disease ^c	Hypoglycaemia ^d	— ^b
Metformin	Renal or liver disease; any predisposition to hypoxia	Gastro intestinal upsets; risk of lactic acidosis if wrongly prescribed	Creatinine, Hb or Vit B12 ^b
Thiazolidinediones	Cardiac failure; liver disease	Oedema, anaemia, heart failure, fractures in women	LFT ^b
α -Glucosidase inhibitors	Chronic intestinal disease	Gastrointestinal upsets	LFT ^{b,e}
Incretin mimetic	Severe renal or gastrointestinal disease	Nausea, hypoglycaemia if used with another antidiabetic agent	— ^b
Gliptins	Severe renal disease	Abdominal pain, hypoglycaemia when used with another antidiabetic agent	Creatinine ^b
Amylin analogue	Gastroparesis	Hypoglycaemia, nausea	— ^b

Hb, haemoglobin; Vit B12, vitamin B12; LFT, liver function test.

^aThe dosage of each antidiabetic drug should be increased until either the target level of glycaemia is achieved or the last dosage increment produces no additional effect.

^bAppropriate monitoring of glycaemic control using fasting or random blood glucose, glycated haemoglobin (HbA1c) or fructosamine (glycated albumin) should be undertaken for all patients receiving antidiabetic drugs.

^cDepending upon pathways of metabolism and elimination of individual members of the class.

^dPrandial insulin releasers are less likely to produce severe or prolonged episodes of hypoglycaemia than sulphonylureas.

^eLiver function should be checked in patients on high dose acarbose.

prandial insulin releaser would be preferred for individuals with either mainly postprandial hyperglycaemia or irregular meal patterns which predispose to interprandial hypoglycaemia when taking a sulphonylurea. An α -glucosidase inhibitor can be used if the hyperglycaemia is modest and predominantly restricted to postprandial periods. The TZDs are slower to take effect than other agents, presumably due to their largely genomic mode of action. In Europe TZDs are used mainly as an alternative to metformin if metformin is not tolerated, or as add-on (combination) therapy with metformin. Nateglinide, or a gliptin (DPP-4 inhibitor) or a GLP-1 analogue (incretin mimetic) are presently recommended as second-line agents to be used in combination with another differently acting agent, when that agent alone does not achieve glycaemic control. The amylin analogue pramlintide is not available in Europe: it is used as add-on therapy to insulin to improve glycaemic control without increasing the insulin dose and without weight gain.

Type 2 diabetes is a progressive disease with continued insulin resistance and gradually declining β -cell function. Thus, hyperglycaemia increases with disease duration and glycaemic control becomes ever more difficult to maintain. If two or possibly three *differently acting* antidiabetic agents listed above do not achieve glycaemic control then it is apposite to switch to insulin therapy (► [insulin](#), [insulin receptor](#)).

The main limitations and precautions for the use of oral antidiabetic drugs are listed in [Table 4](#).

- [Diabetes Mellitus](#)
- [Insulin Receptor](#)
- [Glucose Transporters](#)
- [ATP-dependent K⁺ Channel](#)
- [PPARs](#)

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Antidiarrhoeal Agents

Antidiarrhoeal drugs are used for the symptomatic treatment of diarrhoea (the frequent passage of liquid

faeces). Commonly used antidiarrhoeal drugs are opioids including codeine, diphenoxylate and loperamide. They reduce the motility of the intestine. Other antidiarrhoeal agents (chalk, charcoal, methyl cellulose) probably act by adsorbing toxins or microorganisms causing diarrhoea. Bismuth subsalicylate is used for the treatment of traveller's diarrhoea. It mainly reduces fluid secretion in the bowel.

Antidiuretic Hormone

- [Vasopressin/Oxytocin](#)

Antidysrhythmic Drugs

- [Antiarrhythmic Drugs](#)

Anti-emetic Drugs

- [Emesis](#)

Antiepileptic Drugs

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Definition

► [Epilepsy](#) is a chronic neurological disorder that affects about 0.6–0.8% of the general population worldwide. The clinical hallmark of epilepsy is

recurrent seizures (►seizure), which disrupt normal brain function. A large number of different types of epilepsies and epileptic syndromes have been distinguished. Many specific syndromes start in infancy and are accompanied by further developmental, neuropsychological and metabolic alterations, mostly of unknown origin. Generally, epilepsies with a focal origin (focal epilepsies) are discriminated from epilepsies with a generalized beginning (primary generalized epilepsies).

At the cellular level, focal and general convulsions correspond to synchronized high-frequency ►discharges (►discharge of neurons) of large groups of neurons, which disrupt normal information processing. Depending on the areas of the CNS recruited into the abnormal discharge, clinical symptoms observed during focal seizures may vary considerably. Thus, discharges within limited areas of the motor cortex may lead only to mild motor seizures, while seizure activity in the ►temporal lobe may cause complex semiologies that include behavioral automatisms and loss of consciousness. Focal seizures without loss of consciousness are termed simple partial seizures, whereas focal seizures with loss of consciousness are named complex partial seizures. In some epilepsies, initially focal seizures spread to involve most of the cerebral cortex (secondary generalized seizures).

Primary generalized seizures are also heterogeneous with respect to their clinical features. Such seizures can impose as ►absence epilepsy, which is characterized by a brief interruption of consciousness due to highly synchronized neuronal activity involving thalamocortical networks without increases in neuronal firing rate. On the other hand, ►tonic-clonic convulsions with loss of consciousness are often also primarily generalized.

Basic Mechanisms

Both focal and generalized epilepsies are heterogeneous with respect to their etiology and the principles of therapy.

Basic Mechanisms Underlying Focal Epilepsies

A large group of focal epilepsies arises as a consequence of developmental lesions, CNS tumors, trauma or inflammatory processes, which may be located in neocortical areas as well as the mesial temporal lobe. In a second group of patients, no such causal factor can be identified. Very frequently, such epilepsies arise from a focus within the ►hippocampus, which shows characteristic neuropathological and molecular changes. Only few focal epilepsies seem to be due to a mutation in ion channel ►(ion channels) genes. In contrast, a large number of generalized epilepsies is thought to have a genetic basis, and the chromosomal localization or the

gene mutation has been identified in some of these disorders.

Many patients with focal epilepsies respond well to antiepileptic drugs, but a sizeable portion continues to have seizures even in the presence of optimal therapeutic drug concentrations. For unknown reasons, patients with an epileptic focus residing in the temporal lobe (Temporal Lobe Epilepsy, TLE) often develop pharmacoresistant epilepsy. Therefore, considerable attention has been focused on unraveling the cellular changes underlying hyperexcitability in this form of epilepsy. Identifying such changes is of obvious importance in determining promising novel therapeutic strategies.

In focal epilepsies a number of functional and morphological changes are observed which may act in concert to support enhanced excitability. Such changes have been intensively investigated in order to develop targets for drug design.

- Altered density of voltage-dependent ion currents in neurons: Such changes may considerably affect the firing properties of neurons. They may also affect how neurons integrate a given synaptic input.
- Altered synaptic properties: Numerous changes in the properties of inhibitory (GABAergic) and excitatory (glutamatergic) synapses have been reported. While the simple adage of an imbalance between inhibitory and excitatory neurotransmission in epilepsy is not generally applicable, some forms of inhibition are lost or impaired in epilepsy. Likewise, an increased function of glutamate receptors has been demonstrated in some brain areas.
- Formation of novel aberrant synapses, axonal sprouting: In addition to altered properties of inhibitory and excitatory synapses, numerous synapses are newly formed in chronically epileptic tissue. In some regions, as in the dentate gyrus, the subiculum and area CA1 of the hippocampus, excitatory neurons form recurrent synapses terminating within the same region. This and other forms of recurrent sprouting are thought to constitute a positive feedback pathway facilitating seizure generation in this area. Very little is known about the elementary properties of newly formed synapses.
- Altered properties of glial cells: Glial cells are centrally involved in regulating the size of the extracellular space and the composition of the extracellular milieu, amongst other important tasks. In particular, glial cells normally take up K^+ released by neurons during repetitive neuronal activity. Preventing excessive increases in the extracellular K^+ concentration is important because they may enhance excitability of surrounding neurons. In chronic epilepsy, one of the numerous changes occurring in glial cells is the loss of the capacity to take up K^+ .

Clearly, the largest difficulty in chronic focal epilepsy is to identify amongst the numerous changes that might plausibly affect excitability those that are most important in mediating hyperexcitability. Because of the lack of molecular targets with a proven causal role in mediating seizures, design of anticonvulsant drugs has been driven mainly by considering which drugs potentially limit excitability in normal brain tissue or normal animals. It must be also stressed that, in focal epilepsies, our knowledge extends mainly to the cellular changes that underlie hyperexcitability in the chronic stage of the disease. The factors governing the development of the epileptic condition in humans are much less clear, and the design of substances aimed at inhibiting the progression of epilepsy is in its first stages.

Primary Generalized Epilepsies

Primary generalized epilepsies are a heterogeneous group of diseases. Some of the generalized epilepsies are hereditary, and several genetic mutations of ion channels or membrane receptors linked to this disorder have been identified. In others, the pathogenesis is less clear. Absence epilepsies present with a characteristic 3/s discharge in the electroencephalogram, and the mechanism for similar aberrant discharges have been well studied in animal models. It is thought that thalamic projection neurons that have the capacity to generate burst discharges mediated by low-threshold Ca^{2+} channels provide a phasic excitation of interneurons. These interneurons in turn inhibit thalamic projection neurons via GABA receptors, resulting in a pronounced hyperpolarization. This hyperpolarization removes inactivation of low-threshold Ca^{2+} channels, subsequently enabling these neurons to generate a new, Ca^{2+} channel-dependent burst discharge. Thus, rhythmogenesis seems to rely on the interplay between low-threshold Ca^{2+} channel-dependent bursting and GABA-mediated inhibition. Accordingly, absence epilepsies respond well to substances blocking low-threshold Ca^{2+} channels (ethosuximide, trimethadione), as well as to some GABA-antagonists (which are still in an experimental stage for this indication).

Substances Acting on Voltage-dependent Ion Channels

With few exceptions, information on the anticonvulsant pharmacology of specific ion channel subunits analyzed in expression systems is scarce. Hitherto, a first understanding of the mechanism of action of most antiepileptic drugs has evolved from analyses of somatic ion channel pharmacology either in isolated neurons from human or rodent neurons, or cell culture models.

Voltage-dependent Na^+ Channels

A large number of anticonvulsant drugs commonly in use for focal epilepsies act on fast voltage-dependent

Na^+ channels at clinically relevant concentrations (carbamazepine, phenytoin, lamotrigine). Most of these anticonvulsant drugs display three distinct effects on Na^+ channels:

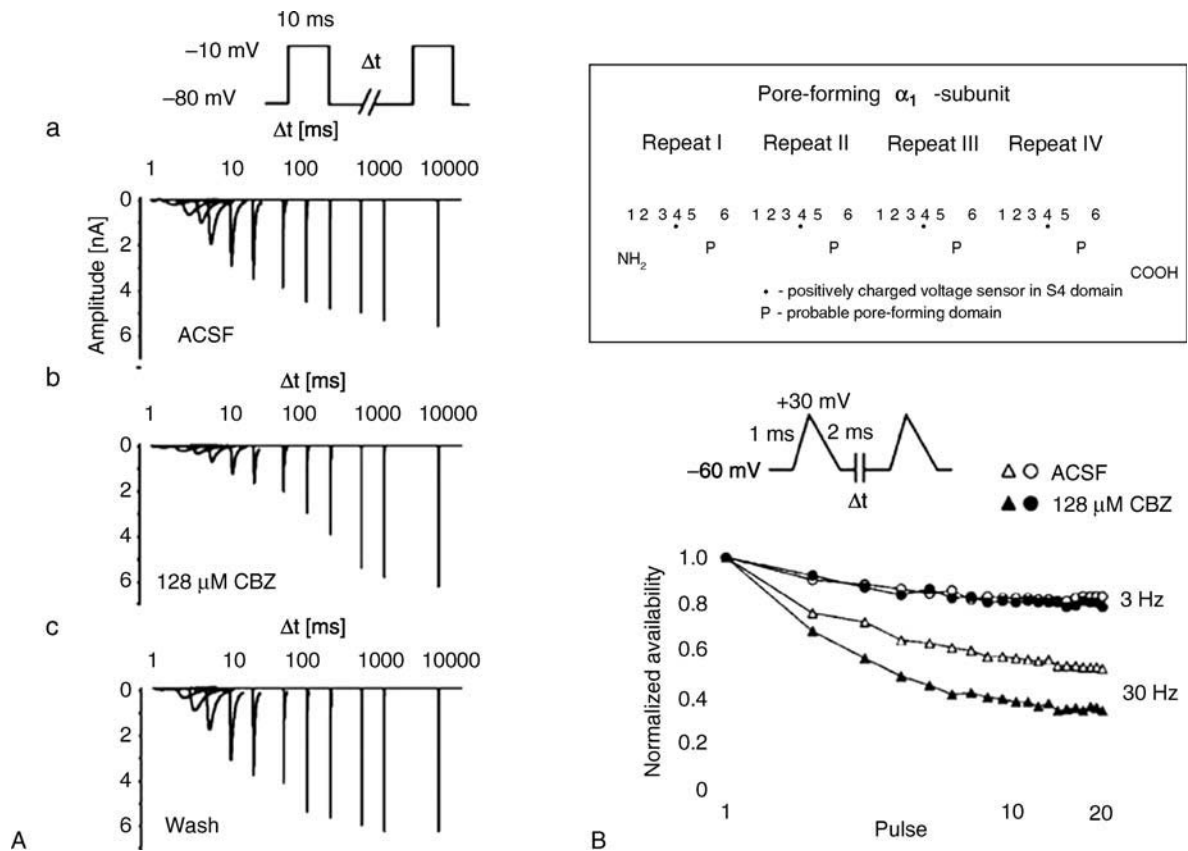
- A shift of the voltage-dependence of inactivation to a hyperpolarizing direction, resulting in a lower fraction of channels available for activation at action potential threshold.
- A reduction of the peak Na^+ channel conductance.
- A pronounced slowing of Na^+ channel recovery from the inactivated state.

The latter effect results in a prolongation of the time required after an action potential for inactivated Na^+ channels to become available again. This prolongation would be expected to inhibit repetitive firing only if the time between action potentials is not long enough to permit recovery of Na^+ channels, i.e. at high discharge frequencies. Indeed, phenytoin, carbamazepine and lamotrigine have been shown to preferentially inhibit high frequency but not low frequency firing (see Fig. 1). It has to be noted that this mechanism is most probably invoked not only at somatodendritic Na^+ channels, but also at presynaptic Na^+ channels. In the latter case, application of one of the antiepileptic drugs mentioned above would be expected to preferentially inhibit transmitter release induced by high frequency presynaptic action potentials.

In addition to inhibiting fast voltage-dependent Na^+ currents, many anticonvulsants also suppress persistent Na^+ currents, in some cases even more efficiently. This mechanism may also be important in the anticonvulsant action of these substances because persistent Na^+ currents are thought to give rise to high frequency burst discharges in some neurons.

Voltage-dependent Ca^{2+} Channels

A number of anticonvulsant drugs also display effects on Ca^{2+} channels. In most cases, effects on Ca^{2+} channels with a depolarized threshold of activation are small at clinically relevant concentrations. In the case of gabapentin, binding to a Ca^{2+} channel accessory subunit has been demonstrated, but whether this binding affects channel function is unknown. In contrast, Ca^{2+} channels with a hyperpolarized threshold of activation (low-threshold channels) are sensitive to a number of drugs (i.e. ethosuximide, trimethadione through its metabolite dimethadione, phenytoin, lamotrigine). As stated above, the activity of ethosuximide and trimethadione against absence epilepsy is thought to be due to their inhibition of low-threshold Ca^{2+} channels. The differing anticonvulsant profile of lamotrigine and phenytoin may be due to the fact that the three pore-forming subunits underlying low-threshold Ca^{2+} channels are differentially sensitive to anticonvulsant drugs.



Antiepileptic Drugs. Figure 1 Effects of carbamazepine on voltage-dependent Na^+ channels. (a) Fast recovery from inactivation can be analyzed in double pulse experiments using the whole-cell patch clamp technique. Recordings shown are from rat hippocampal dentate granule neurons. Inactivation is induced with a conditioning pulse (10 ms, -10 mV), after which recovery of Na^+ channels from inactivation is monitored by a test pulse applied at various intervals following the conditioning pulse (inset, a). Representative traces after various recovery intervals are displayed on an exponential time scale. Application of $128 \mu\text{M}$ CBZ causes a marked slowing of the time course of recovery. (b) Use-dependent block of Na^+ channels by CBZ. Trains of mock action potentials were applied as voltage commands at different frequencies. Application of CBZ reduces Na^+ channel availability preferentially during high-frequency stimulation. Inset: General molecular structure of pore-forming α subunits of voltage-dependent Na^+ channels.

Voltage-dependent K^+ Channels

Up-modulation of voltage-dependent K^+ channels may be a plausible mechanism to reduce cellular excitability and action potential-dependent neurotransmitter release. However, the number of novel antiepileptic drugs developed that target potassium channels is small. Interestingly, it has recently been discovered that a mutation resulting in a moderate loss of function of KCNQ2/3 K^+ channels causes a focal form of epilepsy. The novel anticonvulsant retigabine, which enhances the activity of this very channel type, displays a high clinical efficacy in these patients.

Substances Acting on Neurotransmitter Receptors

A large fraction of anticonvulsants are based on the attempt to boost inhibitory synaptic transmission in

order to restore the balance between inhibition and excitation in epileptic tissue. The first drug using this mechanism of action was phenobarbitone, which was introduced into clinical practice in 1912. Today, there are at least three different targets of anticonvulsant drugs at the synaptic level, all centered on the main inhibitory transmitter GABA (γ -aminobutyric acid).

GABAergic Synapses

Based on the key elements in synaptic inhibitory transmission, three classes of drugs can be distinguished:

- GABA receptor modulators. These substances yield a potentiation of synaptic responses to GABA by changing the affinity of the GABA receptor

(benzodiazepines) or enhancing the open probability of this ligand-gated ion channel (barbiturates). Benzodiazepines are especially useful against status epilepticus but are also used as an adjunctive therapy in partial and generalized seizures. Clinically used substances are clobazepam, clonazepam, clorazepate, diazepam, lorazepam, midazolam and nitrazepam. Barbiturates (esp. phenobarbitone) are used in tonic-clonic and partial seizures, in status epilepticus and in neonatal seizures. Chronic treatment with benzodiazepines and barbiturates is complicated due to the sedative side effects and, most importantly, development of tolerance.

- **GABA-uptake blockers.** Block of GABA-uptake prolongs the presence of the transmitter in the synaptic cleft and thereby strengthens the postsynaptic effects of synaptically released GABA. Tiagabine, a derivative of nipecotic acid, is in clinical use as an add-on therapy against simple and complex partial seizures. Like all substances that generally increase

GABAergic transmission, tiagabine has sedative side effects.

- **Blockers of GABA catabolism.** Blocking the GABA-degrading enzyme GABA-transaminase increases the concentration of GABA in synaptic terminals and enhances or stabilizes the inhibitory transmission. A “new” anticonvulsant designed for this purpose is γ -vinyl-GABA (Vigabatrin), but it should be noted that valproate has the same effect. Vigabatrin is used as an adjunctive therapy in partial and secondary generalized seizures. It is very efficient against infantile spasms and is being used in Lennox-Gastout (together with sodium valproate and benzodiazepines). Side effects of vigabatrin include neuropsychiatric (especially mood) disturbances as well as retinopathic changes. Novel experimental approaches are aimed at increasing GABA synthesis, rather than blocking its degradation, by potentiating the action of the GABA-synthesizing enzyme glutamate decarboxylase.

Antiepileptic Drugs. Table 1 Summary of the known spectrum of actions of a selection of antiepileptic drugs

	Voltage-dependent ion channels			Neurotransmitter receptors			NT release	Other mechanisms
	Na ⁺	Ca ²⁺	K ⁺	GABA	NMDA	AMPA		
Phenytoin	+++	++ (T)	+ (DR)	?	?	?	++	Calmodulin and cyclic nucleotide-dependent second messenger systems
Carbamazepine	+++	—	?	—	+	?	?	Adenosine receptors
Lamotrigine	+++	++	—	—	—	—	?	
Valproic acid	+++	+	+	?	?	?	++	Increase in brain GABA, decrease in brain aspartate
Ethosuximide	+ (I _{Na,p})	+++ (T)	+	?	?	?	—	Inhibition of Na ⁺ /K ⁺ ATPase
Trimethadione	?	+++ (T,L)	?	?	?	?	?	
Phenobarbital	—	+	?	+++	?	?	++	
Diazepam	+	—	—	+++	—	—	—	
Felbamate	++	?	?	?	+++	?	?	
Gabapentin	—	+ (HVA)	+	—	—	—	+	Increased GABA synthesis, GDH, GAD, GABA-aminotransferase
Vigabatrin	?	?	?	?	?	?	+++	GABA-transaminase inhibition
Tiagabine	?	?	?	?	?	?	+++	
Losigamone	?	++	?	++	?	?	?	
Ramecemide	?	?	?	?	?	?	?	
Topiramate	++	?	?	++	—	?	?	
Levetiracetam	—	—(T), + (HVA)	?	?	?	?	?	GABA-T, GAD
Retigabine	?	?	+++ (KCNQ)	?	?	?	?	

This synopsis refers only to actions demonstrated within or close to therapeutic concentrations of drugs. Abbreviations: (+) to (+++) weak to strong efficacy, (—) no efficacy, (?) not investigated. HVA: high threshold Ca²⁺ channels, T: T-type Ca²⁺ channels, L: L-type Ca²⁺ channels, I_{Na,p}: persistent sodium current, DR: delayed rectifier K⁺ channels, KCNQ: KCNQ subtypes of K⁺ channels.

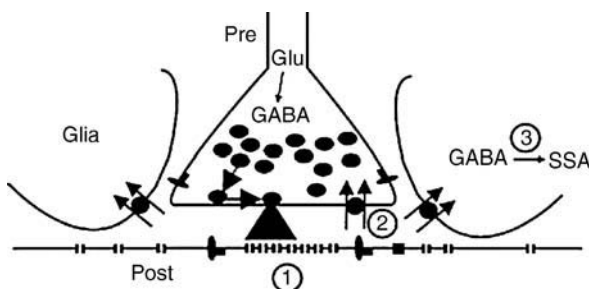
One of the oldest antiepileptic drugs, bromide, has been reported to boost inhibition by an unknown mechanism. Bromide is still in use in certain cases of tonic-clonic seizures and in pediatric patients with recurrent febrile convulsions and others. The mechanism of action may include a potentiation of GABAergic synaptic transmission, although the precise target is not known.

Excitatory Amino Acid Antagonists

The complementary approach to boosting inhibition, i.e. antagonizing the effects of the excitatory neurotransmitter ►glutamate (►GABAergic System), has been less fruitful so far. Antagonists of the NMDA-subtype of glutamate receptors show anticonvulsant activity in animal experiments but have not been introduced into clinical use due to severe neuropsychological side effects. An exception may be felbamate, which seems to exert at least part of its effect by a block on NMDA receptors. Antagonists of two other glutamate receptor subclasses (AMPA- and Kainate-receptors) are under development. Topiramate, a new anticonvulsant drug, partially blocks kainate-receptors and thus may provide the first example of an AED with effects against excitatory neurotransmission.

Substances with Unknown or Mixed Mechanism of Action

It should be pointed out that most anticonvulsants have more than one effect on neuronal excitability or



Antiepileptic Drugs. Figure 2 The GABAergic synapse as a target of anticonvulsant drugs. GABA is synthesized from glutamate in the presynaptic terminal and is packed into small synaptic vesicles. After release, GABA activates postsynaptic ion channels (GABA_AR, marked "1") which mediate the chloride influx and thereby the inhibition of the postsynaptic cell. From the synaptic cleft, GABA is removed into the presynaptic terminal and into a adjacent glia cells by GABA-uptake ("2"). A fraction of the transmitter is degraded into succinyl-semialdehyde by GABA-transaminase ("3"), which is present in glia cells as well as in neurons. Pre- and postsynaptic metabotropic GABA_BR are indicated by ellipsoid bodies in the cell membrane but are no major target for anticonvulsants presently in use. GABAergic drugs against epilepsy act as positive modulators of the GABA_AR ("1"), blockers of GABA-uptake ("2") or inhibitors of GABA degradation ("3").

synaptic transmission. A prominent example is valproic acid, which affects GABAergic transmission (probably by enhancing cellular GABA-content), glutamatergic synaptic transmission by reducing synthesis of excitatory amino acids as well as voltage-dependent ion channels (see Table 1).

Drugs with unknown mechanism of action are gabapentin, bromides (but see above effects on GABAergic transmission) and adrenocorticotrophic hormone (ACTH), which is used in infantile spasms.

- GABAergic System
- Ionotropic Glutamate Receptors
- Voltage-dependent Ca²⁺ Channels
- Voltage-gated K⁺ Channels

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Antiestrogen

Antiestrogens are estrogen/estrogen receptor antagonists.

- Selective Sex Steroid Receptor Modulators
- Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

Antifibrinolytic Drugs

- Fibrinolytics

Antifungal Drugs

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Synonyms

Antimycotic drugs; Antimycotics; Fungicides

Definition

►Fungi cause diseases in plants, animals and humans. Antifungal drugs (fungicides) are therefore used in agriculture, animal and human medicine. In this article, only antifungal drugs used for human chemotherapy are described.

Antifungal drugs inhibit the growth of fungi in tissue (►fungistatic activity) by a number of different mechanisms; some of the agents even kill the fungal cell (►fungicidal effect). Antifungal drugs are used for the treatment of established fungal diseases; however, in immunosuppressed patients at high risk they are also used as prevention or empiric therapy.

Antifungal drugs are classified according to their mode of action and/or their chemical class. Four chemical classes have mainly contributed to the actual armamentary of antifungal drugs: the broadest class is the one of ►azoles (imidazoles and triazoles), followed by ►polyenes, ►allylamines and ►morpholines. Some individual compounds are used in dermatology.

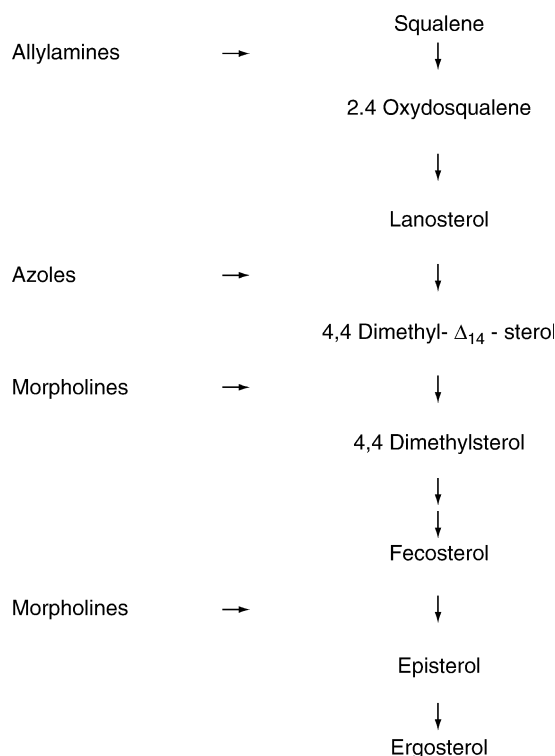
Mechanism of Action

The difficulty of killing the eukaryotic fungal cell without damaging the host is perhaps more akin to the problems of cancer chemotherapy than those of antibacterial treatment. Biochemical studies have identified a number of potential targets for antifungal chemotherapy, including cell wall synthesis, membrane sterol biosynthesis, nucleic acid synthesis, metabolic inhibition and macronuclear biosynthesis. The cell wall synthesis is the only fungal-specific target, since the fungal cell wall has a unique molecular structure; all other pathways (enzymatic steps) in the fungal cell are closely related to the ones used in human cells. (Fig. 1)

Sterol Biosynthesis Inhibitors

Most antifungals on the market (►azoles, ►allylamines, ►morpholines, Tolnaftate, Tolciclate) interfere with the various enzymatic steps involved in the cascade of ergosterol synthesis from squalene to ergosterol; this pathway being the Achilles' heel of the fungal cell. Ergosterol is the essential component of the fungal membrane and exerts two functions: it is the bulk membrane component and it regulates cell growth and proliferation. All sterol biosynthesis inhibitors induce depletion of the essential ergosterol and accumulation of a wrong sterol moiety, consequently disturbing the function of the cell membrane. The morphological changes seen in all cells treated with a sterol biosynthesis inhibitor are similar, all including thickening of the cell wall by chitin deposits.

The imidazoles and triazole (►azoles) (for example, ketoconazole, itraconazole (ITRA), fluconazole (FLU), voriconazole) interfere with cytochrome P₄₅₀-dependent lanosterol C₁₄ demethylase, leading to depletion of ergosterol and accumulation of lanosterol in the



Antiepileptic Drugs. Figure 1 Antifungal drugs.

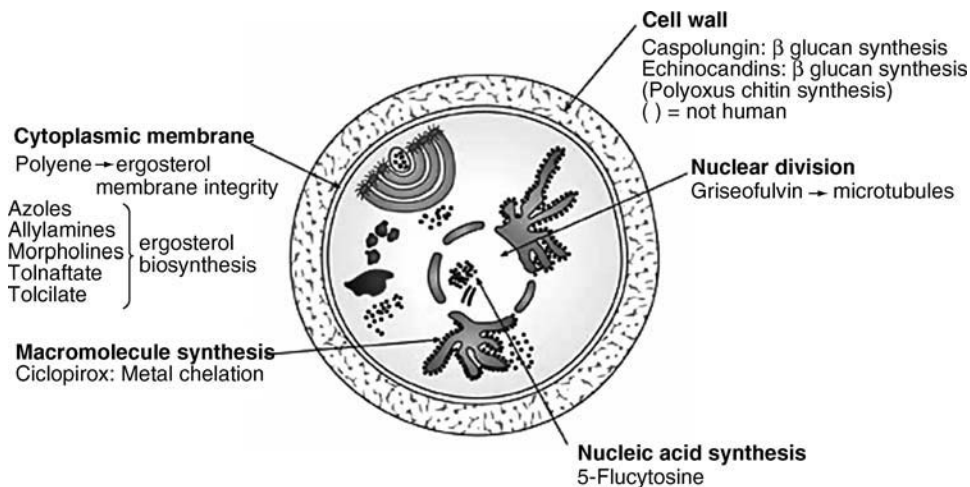
membrane. At the molecular level, one of the nitrogen atoms of the azole ring binds to the haem moiety of cytochrome P₄₅₀. Only compounds with higher specific binding to the fungal cytochrome than to the human one can be used as systemic antifungal drugs. Compared to the imidazoles, the triazoles have a much higher affinity for fungal cytochrome than for human cytochrome P₄₅₀ enzyme steps. In addition to the main interactions with the P₄₅₀ cytochrome, azoles may inhibit cytochrome C oxidase and peroxidative enzymes; they may also interfere with phospholipids. The fact that miconazole and ITRA are fungicidal is thought to be the result of a direct membrane interaction, leading to the loss of cytoplasmic constituents.

Allylamines

Allylamines (terbinafine, naftifine) interfere with the ergosterol pathway at the level of squalene epoxidase leading to the depletion of ergosterol and the accumulation of squalene. Again, only compounds with a higher specificity for the fungal enzyme than for the human enzyme can be used for systemic use. A clear correlation exists between growth inhibition and degrees of sterol biosynthesis inhibition; the fungicidal effect is more correlated to the intracellular accumulation of squalene. (Fig. 2).

Morpholines

Morpholines (amorolfine) interfere at two levels of the ergosterol pathway, the Δ₁₄-reductase and the Δ₇-,



Antiepileptic Drugs. Figure 2 Antifungal drugs – targets of antifungal activity.

Δ_8 -isomerase leading to depletion of ergosterol and accumulation of an unplanar sterol *ignosterol*. With the inhibition of two steps in the same pathway, a natural synergistic effect is built into the molecule so that the risk of appearance of resistant mutants is low and efficacy high.

Drugs Binding Directly to Ergosterol (amphotericin B, nystatin, candicidin).

The current model for the mechanism of the ►**polyene** amphotericin B (Amph B) is based on the formation of a 1:1 Amph B/Ergosterol aggregate, which associates into a transmembrane channel with a large –OH lined aqueous pore down the middle. The result of the interaction between Amph B and the sterols is the disturbance of the ergosterol function leading to increased permeability, disruption of the proton gradient and leakage of potassium. The fungicidal effect, however, has been linked to irreversible inhibition of the membrane ATPase.

5-Fluorocytosine

5-Fluorocytosine (5FC), a mock pyrimidine, is the only antifungal drug that acts as true antimetabolite. 5FC is taken up into the fungal cell, deaminated to 5-fluorouracil (5FU) which is the active principle responsible for the killing of the fungal cell. Fungi lacking the cytosine deaminase are resistant to 5FC. Intracellularly, 5FU acts along two different pathways: it is incorporated as 5-fluorouridine monophosphate into the RNA and it inhibits after conversion to 5-fluorodeoxyuridine monophosphate, the thymidylate synthetase, leading to inhibition of DNA synthesis. 5FU itself cannot be used for antifungal therapy due to its toxicity for mammalian cells.

Glucan Synthase Inhibitors

Echinocandins (i.e. caspofungin), semisynthetic lipopeptides, inhibits the synthesis of β -(1,3)-D-glucan, an

integral component of the fungal cell wall not present in mammalian cells.

Griseofulvin

Griseofulvin is the first antimycotic drug detected that is only active against ►**dermatophytes**. Its activity manifests as nuclear and mitotic abnormalities followed by distortions in the hyphal morphology.

Hydroxypyridones (Ciclopirox, Rilopirox)

The primary mode of action of this class of antimycotics is interference with uptake and accumulation of products required for cell membrane synthesis. In higher concentrations it causes a disturbance of the cellular permeability. Some investigations show an interaction with Fe(III)- ions; the compounds acting as chelators. Very high concentrations interfere with the function of fungal mitochondria.

Clinical Use (including Side Effects)

Fungal diseases divide themselves into three classes: superficial (topical, local) mycoses (►**dermatomycoses** and gynaecological infections), subcutaneous and organ mycoses. This division is important not especially for microbiological reasons, but in the view of the different problems arising during treatment. Superficial mycoses are not life threatening, but they are irritating. Subcutaneous mycoses are also not life threatening but are associated with a high morbidity, and deep mycoses, especially in immunosuppressed patients, are life threatening showing a high mortality rate in patients, if untreated. The treatment schedules (dose, duration of treatment, galenical formulation) are strongly dependent on the localisation of the fungal disease, on the pathogenicity of the fungi, and on the conditions of the host. Additionally, the diagnosis of the disease is not always guaranteed; therefore, a clear-cut, simple description of clinical usage for antifungal drugs is not possible.

Due to the divergence of fungal diseases, there is neither single best treatment nor a superior drug for all diseases. However, a superior drug does exist for dermatomycoses caused by dermatophytes, namely the allylamine terbinafine (TER). For the treatment of deep mycoses in immunosuppressed patients the most efficacious drug is the polyene Amph B.

The therapy of ▶dermatomycoses and acute vaginal infections is unproblematic. A large choice of various drugs (all chemical classes and compounds discussed above) in different galenical formulations (crèmes, tinctures, sprays, ovula, powder, shampoo, nail lacquer and tablets) exists. All drugs topically applied – used in various treatment schedules – show high efficacy and a low incidence of adverse reactions. For the treatment of onychomycoses without matrix involvement two nail lacquers (a morpholine and hydroxypyron) are on the market showing high efficacy with low (<1%) adverse reactions after topical therapy for several months. A combination therapy with a topical and a systemically applied antifungal drug is the most efficacious and the most economic therapy for onychomycosis with matrix involvement. Systemic therapy is indicated if the dermatomycosis is widespread. The highest cure rate is achieved with TER. TER is well tolerated in adults and children. In ca. 5% of the patients, mild and reversible side effects have been observed: gastrointestinal, skin, central nervous system, respiratory events and loss of taste. For acute vaginal candidosis the treatment of choice with the highest compliance and best efficacy is one daily dose of FLU.

Three fungal infections – Madura feet (mycetoma), chromomycosis and ▶sporotrichosis – fall into the category of subcutaneous mycoses, their distribution is mainly in tropical and subtropical areas. The ideal treatment for madura feet caused by fungi is not yet established; the azoles are of some benefit, however, neither the optimal drug, dose, nor the treatment schedules are known. Chromomycosis responds well to ITRA monotherapy or the combination of 5FC plus ITRA. ITRA has been set up as standard therapy for cutaneous and lymphatic sporotrichosis.

Systemic Mycoses

Systemic mycoses are caused either by true pathogenic fungi (endemic in distinct areas of USA/South America) or by opportunistic fungi that induce severe infections in immunosuppressed patients. The arsenal for the treatment of deep organ mycoses is relatively small: Amph B, 5FC, azoles (FLU, ITRA, voriconazole (NDA filing)) and CAS.

The polyene Amph B (intravenous formulation) has the broadest spectrum, is fungicidal and shows its superiority in immunosuppressed patients. Its only drawback is its infusion-related toxicity and its negative influence on renal function. Acute reactions to Amph B – usually fever chills, rigor and nausea – can be

ameliorated by concomitant administration of meperidine, acetomiophen or hydrocortisone. Additionally, there is the possibility of tailoring time and duration of infusion. Prevention of the chronic tubular injury is feasible by salt loading. Encapsulation of Amph B into liposomes or complexing of the drug with other lipid carriers brings a major reduction of nephrotoxicity. Three lipid-associated forms are now available (Ambisome, Amph B lipid complex and Amph B colloidal dispersion). Due to its toxic side effects, Amph B is not widely used for prevention; it is, however, often used as empiric therapy with high success rates.

Due to the rapid appearance of resistance, 5FC is only used as a combination partner for the intensive therapy of established severe fungal infections caused by *Candida spp.*, *Cryptococcus neoformans* and *Aspergillus sp.* Anorexia, nausea, vomiting, diarrhoea and or abdominal pain occur in 6% of the patients. Of greater concern is the potential for bone marrow depression (seen in 5% of the patients, all with elevated 5FC levels).

Azoles

Generally the azoles are well tolerated in children and adults; mild side effects like nausea and vomiting are seen in <5% of the patients treated with FLU.

Attention has to be given to the problem of interaction between azoles and other drugs; these are based on two mechanisms:

- inhibition of absorption of the azoles leading to lower bioavailability or
- interference with the activity of hepatic microsomal enzymes, which alters the metabolism and plasma levels of azole, the interacting drug or both. This latter induces often increased toxicity of the concomitant drug.

With FLU, only few drug interactions are seen, namely with rifampicin (reduction of FLU), phenytoin, cyclosporin, tolbutamide and warfarin (increasing levels of concomitant drug). The interactions with ITRA are more significant than with FLU: H₂ antagonists and all drugs increasing intragastric pH decrease the absorption of ITRA. Interactions due to hepatic enzymes are seen with rifampicin (reducing the levels of ITRA to undetectable levels), phenytoin, isoniazid, carbamazepine, phenobarbital, midazolam, triazolam, digoxin, lovastatin terfenadine, warfarin and cyclosporin. The list of interacting drugs is still increasing.

Oral FLU is well established as first line therapy for oropharyngeal candidosis and *Candida* oesophagitis and for maintenance therapy in AIDS patients with meningeal cryptococcosis. FLU (oral or intravenous) is also efficacious in candidemia without neutropenia. It shows efficacy in prevention (attention: *Aspergillus sp.* are not in the spectrum) and empiric therapy. ITRA, being fungicidal against *Aspergillus sp.*, shows

promising results in aspergillosis, especially under intravenous therapy, and is used as maintenance therapy in AIDS patients with histoplasmosis. ITRA is the first line therapy for histoplasmosis and blastomycosis in HIV-negative patients. A combination of ITRA plus 5FC may be the optimal therapy of phaeohyphomycoses.

The glucan synthase inhibitor caspofungin (intravenous formulation) is new on the market for the treatment of invasive aspergillosis in patients whose disease is refractory to, or who are intolerant of, other therapies. During the clinical trials fever, infused vein complications, nausea, vomiting and in combination with cyclosporin mild transient hepatic side effects were observed. Interaction with tacrolimus and with potential inducer or mixed inducer/inhibitors of drug clearance was also seen.

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Antigen

An antigen is a molecule recognised by specific receptors on cells of the immune system such as B lymphocytes.

- Immune Defense
- Humanized Monoclonal Antibodies

Antigen Presentation

Antigen presentation is the key mechanism allowing T-lymphocytes to survey whether intracellular pathogens exist in the cells of the body. As T-lymphocytes can only recognize antigen in the form of peptides presented on specific molecules termed major histocompatibility complex (MHC), antigen-presenting cells instruct T-cell reaction and thus the development of adaptive immunity. Professional antigen-presenting

cells (MHC class II positive) are dendritic cells, monocytes/macrophages, and B-lymphocytes.

- Immune Defense

Antigen-presenting Cells

Antigen-presenting cells (APCs) are cells of the immune system that are able to process and present foreign antigens to effector cells. The antigen is presented in the context of an MHC-I or MHC-II molecule on APCs in the presence of so-called co-stimulatory molecules to activate the effector cells.

- Immune Defense
- DNA Vaccination and Genetic Vaccination

Antigen Receptors

Each T- and B-lymphocyte carries one type of receptor, which recognizes one specific antigen. T-cells carry the heterodimeric T-cell antigen receptor consisting of an alpha and beta chain. This receptor recognizes a peptide presented by a MHC (major histocompatibility complex) molecule. B-cells express an immunoglobulin on their surface, which can recognize epitopes on antigens of different sizes and qualities without the need for presentation. Both forms of antigen receptors are created by random genetic rearrangement during the ontogeny of each individual lymphocyte. Both types of antigen receptors require additional transmembrane molecules for signal transduction.

- Immune Defense

Anti-gout Drugs

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Synonyms

Drugs for the treatment/management of ►gout and/or ►hyperuricemia

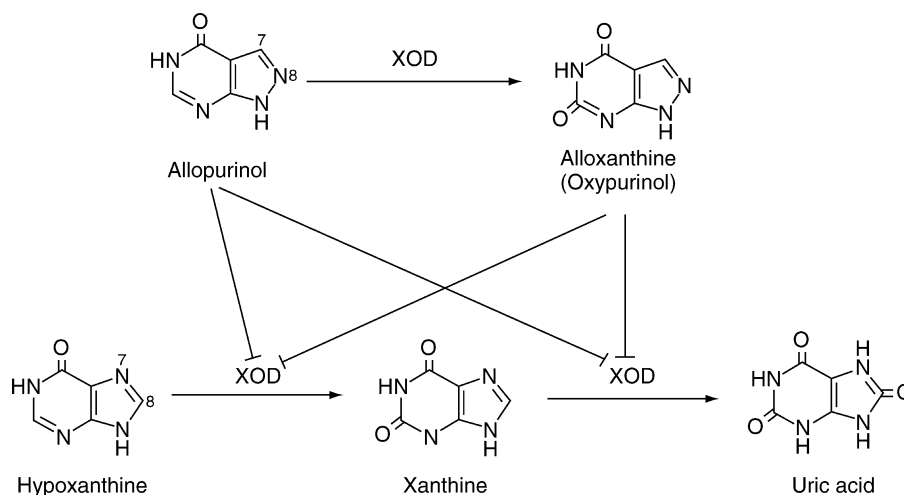
Definition

Pathophysiology and Clinical Manifestations of Gout

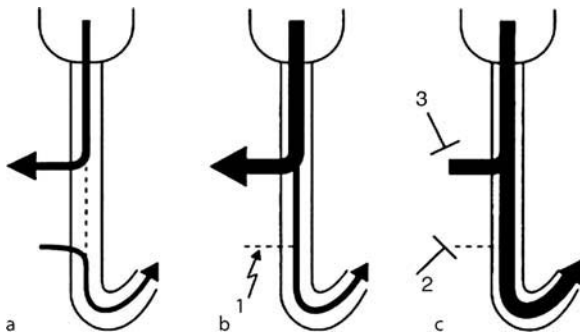
►Uric acid is the end product of purine catabolism in man. Purines originate from food and the degradation of nucleic acids and nucleotides. ►Xanthine oxidase (XOD) is the key enzyme in purine degradation. XOD converts hypoxanthine to xanthine, and xanthine to uric acid, respectively (Fig. 1). Uric acid is filtered in the glomerulus of the kidney, is almost completely absorbed in the proximal tubules and secreted more distally (Fig. 2). At physiological pH (<7.4), uric acid exists predominantly in its ionic form (urate). At lower pH, the fraction of uric acid molecules (protonized form) increases. This is important because uric acid possesses a lower solubility than urate. Thus, a decrease in pH, as it occurs in inflamed tissue and in the tubules, facilitates the formation of uric acid crystals, which are the initial cause of gout. In most mammals, the enzyme uricase converts uric acid to the more soluble allantoin, but humans do not express uricase. Of importance for therapeutic intervention is the fact that xanthine and hypoxanthine are more soluble than uric acid. Specifically, by preventing uric acid formation through XOD inhibition, the excretion of xanthine and hypoxanthine increases, and the risk of uric acid crystal formation decreases. An increase of the serum uric acid concentration above $416 \mu\text{mol/L}$ is referred to as hyperuricemia and is associated with an increased risk of uric acid crystal formation and acute attacks of gouty arthritis. With a serum uric acid level of $535 \mu\text{mol/L}$, the annual incidence of gouty arthritis is 4.9–5.7%.

Hyperuricemia can have genetic causes or acquired causes. A defect of hypoxanthine-guanine phosphoribosyl transferase is the cause of Lesch–Nyhan syndrome, resulting in increased uric acid production. Among the genetically caused defects, impaired renal uric acid secretion is a very common cause of gout. Myeloproliferative diseases, a purine-rich diet (e.g. meat, beer, beans, peas, oatmeal, or spinach), obesity, and alcoholism are common causes of acquired hyperuricemia and result from increased uric acid production. Renal diseases and the application of certain drugs such as the tuberculostatic drug pyrazinamide, thiazide diuretics, loop diuretics, or acetylsalicylic acid at doses of up to 1–2 g/day, and the immunosuppressant cyclosporin A are acquired causes of impaired uric acid secretion.

Gout is the consequence of hyperuricemia and is caused by uric acid deposits in joints, tendons, bursae, kidney and urinary tract. In the USA, the prevalence of gout is ~1% for all ages and both sexes. The prevalence of gout is higher in men than in women and exceeds 5% in men ≥ 65 years. These epidemiologic data are important for drug therapy since older patients are more sensitive to side effects of anti-gout drugs than younger patients. In the initial stage, gout is characterized by asymptomatic hyperuricemia. In the second stage, the disease manifests itself by acute gouty arthritis. The third (intercritical) stage is asymptomatic, and the fourth stage is characterized by progressive uric acid deposits in joints, tendons, bursae, kidney, and urinary tract (tophus formation). Uric acid deposits result in the deformation and loss of function of joints



Anti-gout Drugs. Figure 1 Xanthine oxidase-catalyzed reactions. Xanthine oxidase converts hypoxanthine to xanthine and xanthine to uric acid, respectively. Hypoxanthine and xanthine are more soluble than uric acid. Xanthine oxidase also converts the uricostatic drug allopurinol to alloxanthine. Allopurinol and hypoxanthine are isomers that differ from each other in the substitution of positions 7 and 8 of the purine ring system. Although allopurinol is converted to alloxanthine by xanthine oxidase, allopurinol is also a xanthine oxidase inhibitor. Specifically, at low concentrations, allopurinol acts as a competitive inhibitor, and at high concentrations it acts as a noncompetitive inhibitor. Alloxanthine is a noncompetitive xanthine oxidase inhibitor. XOD: xanthine oxidase.



Anti-gout Drugs. Figure 2 Reabsorption and secretion of uric acid in the proximal renal tubulus. (a) Normal situation. Uric acid is completely reabsorbed in the proximal segment of the renal tubulus and secreted more distally. (b) Situation in untreated hyperuricemia. In most genetically caused cases of gout, uric acid secretion is defective (1). (c) Situation in hyperuricemia under treatment with uricosuric drugs (2); Inhibition of uric acid secretion by uricosuric drugs at low doses (3). Inhibition of uric acid secretion and reabsorption by uricosuric drugs in therapeutic doses. The inhibition of uric acid secretion with low doses of uricosuric drugs can further increase blood levels of uric acid and induce attacks of acute gouty arthritis.

and recurrent episodes of urate lithiasis. Uric acid deposits in the kidney and urate lithiasis can ultimately result in renal failure.

Figure 3 illustrates important pathophysiologic events leading to acute gouty arthritis. Once the concentration of uric acid exceeds its solubility, uric acid crystals form in the synovial fluid of joints. Subsequently, the uric acid crystals are phagocytosed by synoviocytes that form the inner cell layer of joints. Next, synoviocytes release numerous mediators of inflammation including leukotriene B₄ (LTB₄), prostaglandin E₂ (PGE₂), platelet-activating factor (PAF), histamine, interleukins (ILs) 1, 6, and 8, and tumor necrosis factor- α that in conjunction with products of the complement cascade (C5a and C3a) and kinins (bradykinin) induce an inflammatory response. Moreover, LTB₄, PAF, C5a, and IL-8 attract polymorphonuclear leukocytes (neutrophils). Neutrophils migrate into affected joints along a concentration gradient of these inflammatory mediators (chemotaxis). Accordingly, LTB₄, PAF, C5a, and IL-8 are also referred to as chemoattractants. Once present in joints, neutrophils phagocytose uric acid crystals. Uric acid crystals and chemoattractants trigger the release of cytotoxic lysosomal enzymes, NADPH oxidase-catalyzed formation of reactive oxygen species, LTB₄ formation and the release of other proinflammatory molecules from neutrophils. The latter molecules attract additional neutrophils and mononuclear phagocytes. Moreover, neutrophils generate lactate that decreases the pH within the joint and further accelerates uric acid crystal formation. Oxygen radicals and lysosomal enzymes cause damage to tissues.

Thus, the presence of uric acid crystals in joints triggers a vicious cycle, resulting in an extremely painful inflammation. A typical localization of acute gouty arthritis is the first metatarsal joint of the foot (podagra). The diagnosis of acute gouty arthritis is confirmed by the detection of urate crystals in the joint or tophus.

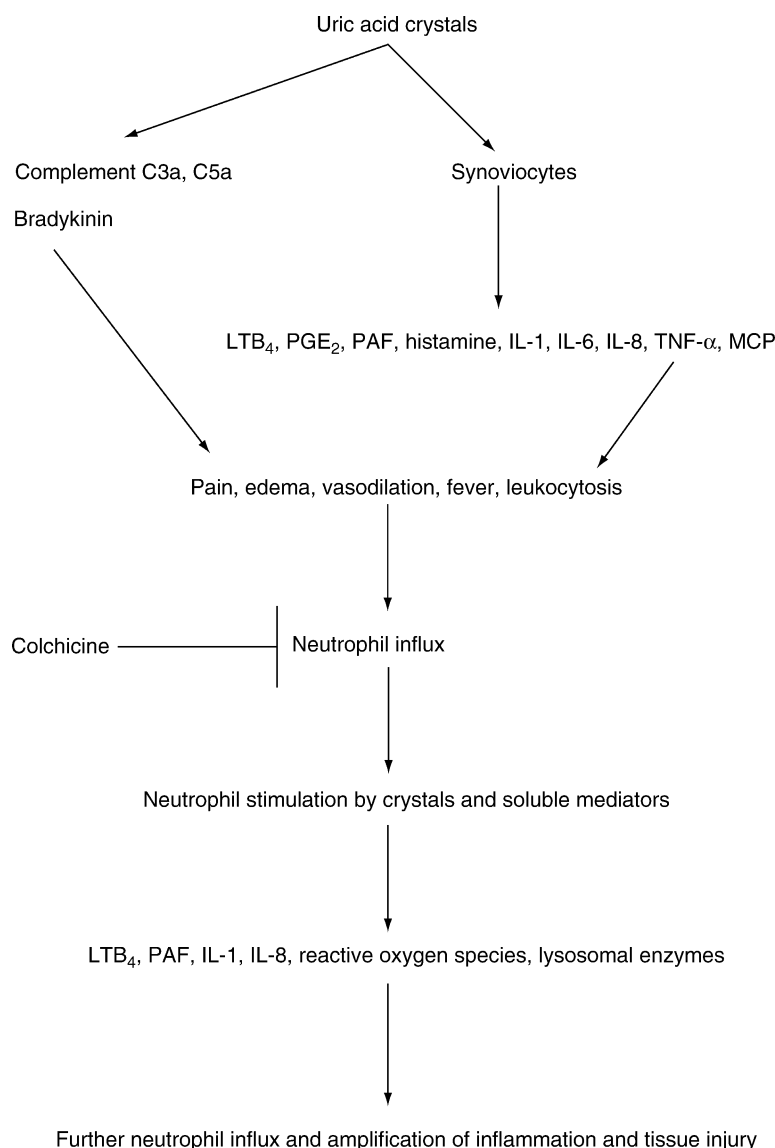
Anti-gout Drugs

Figure 4 shows the structures of commonly employed anti-gout drugs. The treatment of acute gouty arthritis aims at rapidly reducing the pain and inflammatory reaction. This aim can be achieved by treatment with colchicine. In addition, **nonsteroidal anti-inflammatory drugs** (NSAIDs), **glucocorticoids**, and adrenocorticotrophic hormone (ACTH) can be used to treat acute gouty arthritis. However, since NSAIDs and glucocorticoids are used in numerous other commonly occurring inflammatory conditions, they are not *per se* considered specific anti-gout drugs. Glucocorticoids can be given systemically (orally, intramuscularly, or intravenously) or locally into afflicted joints. The long-term goals of gout treatment are the prevention of acute gouty arthritis, the prevention of urate lithiasis and renal failure and the resorption of existing uric acid deposits in the joints and urinary tract. The long-term therapy aims at reducing the serum concentration of uric acid below 357 $\mu\text{mol/L}$. Therapy with the **uricostatic drug** allopurinol and the **uricosuric drugs** benzbromarone, sulfinpyrazone, or probenecid can accomplish the long-term goals. These drugs are well tolerated in most patients. Uricostatic and uricosuric drugs can be combined. Additionally, low doses of colchicine can be used to prevent the occurrence of acute gouty arthritis. However, as is unfortunately often the case with classic diseases, there are only few well conducted clinical studies assessing the clinical efficacy and safety of anti-gout drugs.

Mechanism of Action

Colchicine

Colchicine is an alkaloid from the autumn crocus *Colchicum autumnale*. Colchicine binds to the cytoskeletal protein **tubulin** and, thereby, prevents microtubule formation. As a result, colchicine inhibits neutrophil chemotaxis and the influx of these cells into areas containing uric acid crystals (**Fig. 3**). Colchicine also inhibits neutrophil phagocytosis. As a result, colchicine interrupts the vicious cycle of inflammation in gouty arthritis. However, because of its mechanism of action, colchicine is most effective only when given in the early stages of gouty arthritis, i.e. within 24 h. Otherwise, the inflammatory reaction may be too advanced. Specifically, colchicine is effective in >90% of patients when given within the first few hours after the start of the attack, but after 24 h, the responsiveness decreases to 75%. Given the very significant side effects of colchicine, it is absolutely crucial to initiate colchicine therapy as early as possible.



Anti-gout Drugs. Figure 3 Important pathophysiologic events in acute gouty arthritis. Uric acid crystals activate the complement cascade, the formation of kinins and the release of various mediators of inflammation from synoviocytes that phagocytose uric acid crystals. The combined action of the released mediators induces a strong inflammatory reaction that is further enhanced by neutrophils. Neutrophils migrate along a concentration gradient to loci in which C5a, LTB₄, PAF and IL-8 are produced (chemotaxis). Accordingly, C5a, LTB₄, PAF and IL-8 are also referred to as chemoattractants. Neutrophils phagocytose uric acid crystals. Upon exposure to uric acid crystals and chemoattractants, neutrophils release various mediators of inflammation, reactive oxygen species and lysosomal enzymes. The concerted effects of all these compounds amplify the inflammatory reaction even further. Colchicine interrupts the vicious cycle of inflammation predominantly by inhibiting neutrophil chemotaxis. IL-1, interleukin 1; IL-6, interleukin 6; IL-8, interleukin 8; LTB₄, leukotriene B₄; MCP, monocyte chemoattractant protein; PAF, platelet-activating factor; PGE₂, prostaglandin E₂; TNF-α, tumor necrosis factor-α.

Allopurinol

Allopurinol is an analog of hypoxanthine and is converted to alloxanthine by XOD. Both allopurinol and hypoxanthine inhibit XOD (Fig. 1). Alloxanthine is a noncompetitive inhibitor of XOD as is allopurinol at high concentrations. At low concentrations, allopurinol is a competitive inhibitor of XOD. As a result of XOD inhibition, the formation of the poorly soluble

uric acid is reduced, whereas the formation of the more soluble metabolites hypoxanthine and xanthine is increased. Because of the good solubility of hypoxanthine and xanthine, formation of hypoxanthine/xanthine crystals is a rare complication of allopurinol treatment. Another consequence of XOD inhibition is the accumulation of the precursor of xanthine, inosine. Inosine inhibits the key enzyme of *de novo* purine synthesis,

phosphoribosyl-pyrophosphate amidotransferase. The allopurinol metabolite allopurinol ribonucleotide also inhibits phosphoribosyl-pyrophosphate amidotransferase. Inhibition of purine biosynthesis contributes to the anti-hyperuricemic effects of allopurinol.

Uricosuric Drugs

Depending on the dose applied, uricosuric drugs inhibit tubular reabsorption and tubular secretion of uric acid in the kidney differentially (Fig. 2). At low (subtherapeutic) doses, uricosuric drugs inhibit uric acid secretion without inhibiting reabsorption. Therefore, low doses of uricosuric drugs can actually increase serum levels of uric acid and trigger acute attacks of gouty arthritis. At higher, i.e. therapeutic doses, uricosuric drugs inhibit both tubular secretion and tubular reabsorption. Since inhibition of tubular reabsorption is quantitatively more important than inhibition of tubular secretion, the net effect is an increased renal elimination of uric acid. In order to avoid formation of uric acid crystals in the kidney and urinary tract, it is important that the pH of the urine is kept >6.0 . This goal can be achieved by the oral administration of potassium sodium hydrogen citrate, sodium bicarbonate or acetazolamide. In addition, it is mandatory that the patient drinks at least 3L per day to avoid formation of uric acid crystals.

New Drugs

Rasburicase is a recombinant urate oxidase that catalyzes the conversion of uric acid to allantoin which possesses a greater water-solubility than uric acid. In contrast to allopurinol, rasburicase has also an inhibitory effect on

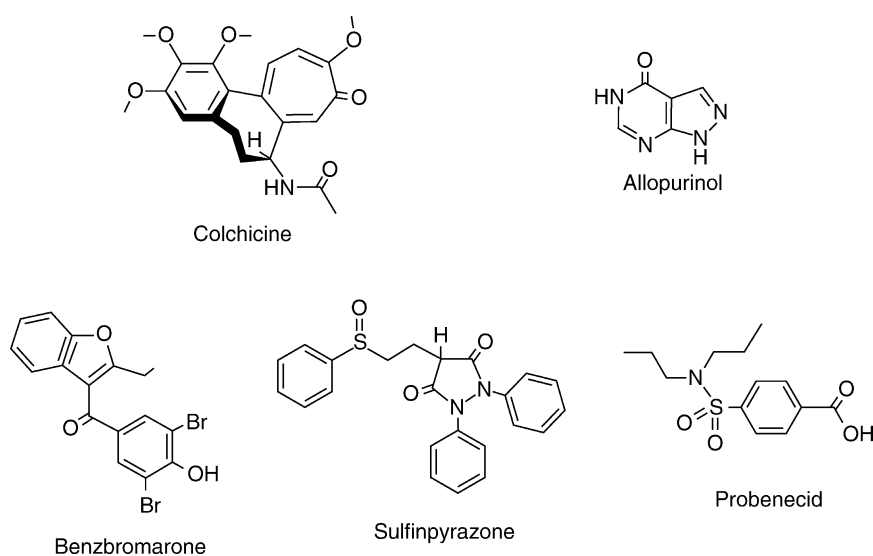
existing uric acid pools, and therapy with the enzyme does not require urine alkalinization. Rasburicase is used in the treatment of pediatric patients with leukemias, lymphomas, and solid tumors who are at high risk for chemotherapy-induced hyperuricemia. Hyperuricemia is part of the so-called tumor lysis syndrome. Further studies are required to compare the clinical efficiency of rasburicase in adult patients and its efficiency in comparison to standard treatment with allopurinol. The use of rasburicase can be associated with severe side effects including hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency, methemoglobinemia, kidney failure, and anaphylactic reactions.

Febuxostat (TEI-6720, TMX-67) is a highly potent mixed-type inhibitor of XOD with K_i values in the 0.5–3 nM-range. Febuxostat displays high selectivity relative to a large number of other purine- and pyrimidine-metabolizing enzymes. Febuxostat can be administered orally and is well tolerated, and there is no need for dose-adjustment in patients with kidney failure and mild-to-moderate liver failure. Therapeutic doses are 80–120 mg daily. Clinical trials indicate that febuxostat may be superior to allopurinol at reducing serum urate concentrations but not at reducing the incidence of gouty arthritis and tophus formation.

Clinical Use (including side effects)

Colchicine

Daily doses of 3–8 mg (6–8 times 0.5–1.0 mg) are used for the treatment of acute gouty arthritis. For prophylaxis, daily doses of 0.5–1.5 mg are used, but the use of colchicine for prophylaxis is controversial.



Anti-gout Drugs. Figure 4 Structures of commonly used anti-gout drugs. Colchicine is an alkaloid from the autumn crocus *Colchicum autumnale* and inhibits tubulin polymerization. Allopurinol is an isomer of xanthine and inhibits uric acid formation (uricostatic drug). Benzbromarone, sulfipyrazone and probenecid are uricosuric drugs and inhibit uric acid reabsorption in the proximal tubulus of the kidney.

The side effects of colchicine are very significant. About 80% of the patients experience gastrointestinal problems including nausea, vomiting, and diarrhea. The anti-mitotic effects of colchicine can result in thrombocytopenia, agranulocytosis, hair loss, and azoospermia. In the central nervous system, confusion, ascending paralysis, respiratory failure, and seizures have been reported. These side effects can be explained by the fact that intact microtubules are essential for proper transport functions in neuronal axons. Moreover, colchicine can cause myopathy. Because of the significant side effects, many physicians prefer to treat acute gouty arthritis with NSAIDs or glucocorticoids. Although colchicine is a classic anti-gout drug, colchicine can also be used to treat other inflammatory diseases including amyloidosis, Dupuytren's contracture, Behcet's syndrome, vasculitis, various forms of hepatic cirrhosis, pulmonary fibrosis, pericarditis, and various inflammatory diseases of the skin. Colchicine is extensively metabolized through the hepatic cytochrome CYP 3A4. Accordingly, inhibitors of CYP 3A4 such as diltiazem, gestodene, grapefruit juice, ketoconazole, and macrolide antibiotics prolong and enhance the pharmacological (and toxic) effects of colchicine. Drugs that are inactivated via CYP 3A4 such as steroid hormones, lidocaine, midazolam, quinidine, terfenadine, nifedipine, and verapamil can also prolong colchicine action. Because of its anti-mitotic effects, colchicine should not be used in pregnant women.

Allopurinol

The daily dose of allopurinol is 300–600 mg. In combination with benzbromarone, the daily allopurinol dose is reduced to 100 mg. In general, allopurinol is well tolerated. The incidence of side effects is 2–3%. Exanthems, pruritus, gastrointestinal problems, and dry mouth have been observed. In rare cases, hair loss, fever, leukopenia, toxic epidermolysis (Lyell syndrome), and hepatic dysfunction have been reported. Allopurinol inhibits the metabolic inactivation of the cytostatic drugs azathioprine and 6-mercaptopurine. Accordingly, the administered doses of azathioprine and 6-mercaptopurine must be reduced if allopurinol is given simultaneously.

Uricosuric drugs

Benzbromarone

The daily dose of benzbromarone is 50–200 mg. In combination with allopurinol, the benzbromarone dose is reduced to 20 mg. Benzbromarone is well tolerated. Rare side effects are headaches, gastrointestinal problems, and exanthems.

Probenecid

The daily dose of probenecid is 0.5–3.0 g. Probenecid is well tolerated, and there are few serious side effects. In

less than 10% of the treated patients, gastrointestinal disturbances, hypersensitivity, and skin reactions occur.

Sulfinpyrazone

The daily dose of sulfinpyrazone is 200–400 mg. The side effects of sulfinpyrazone are comparable with those of probenecid. A potential therapeutic advantage of sulfinpyrazone in patients with coronary heart disease and thromboembolic diseases is its inhibitory effect on platelet aggregation.

► Inflammation

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Antihistamines

The term antihistamines describes drugs which bind to the H₁-histamine receptor and antagonize (block) the histamine effect in Type I allergic responses.

► Allergy

► Histaminergic System

Antihypertensive Drugs

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Synonyms

Antihypertensives; Blood pressure lowering drugs

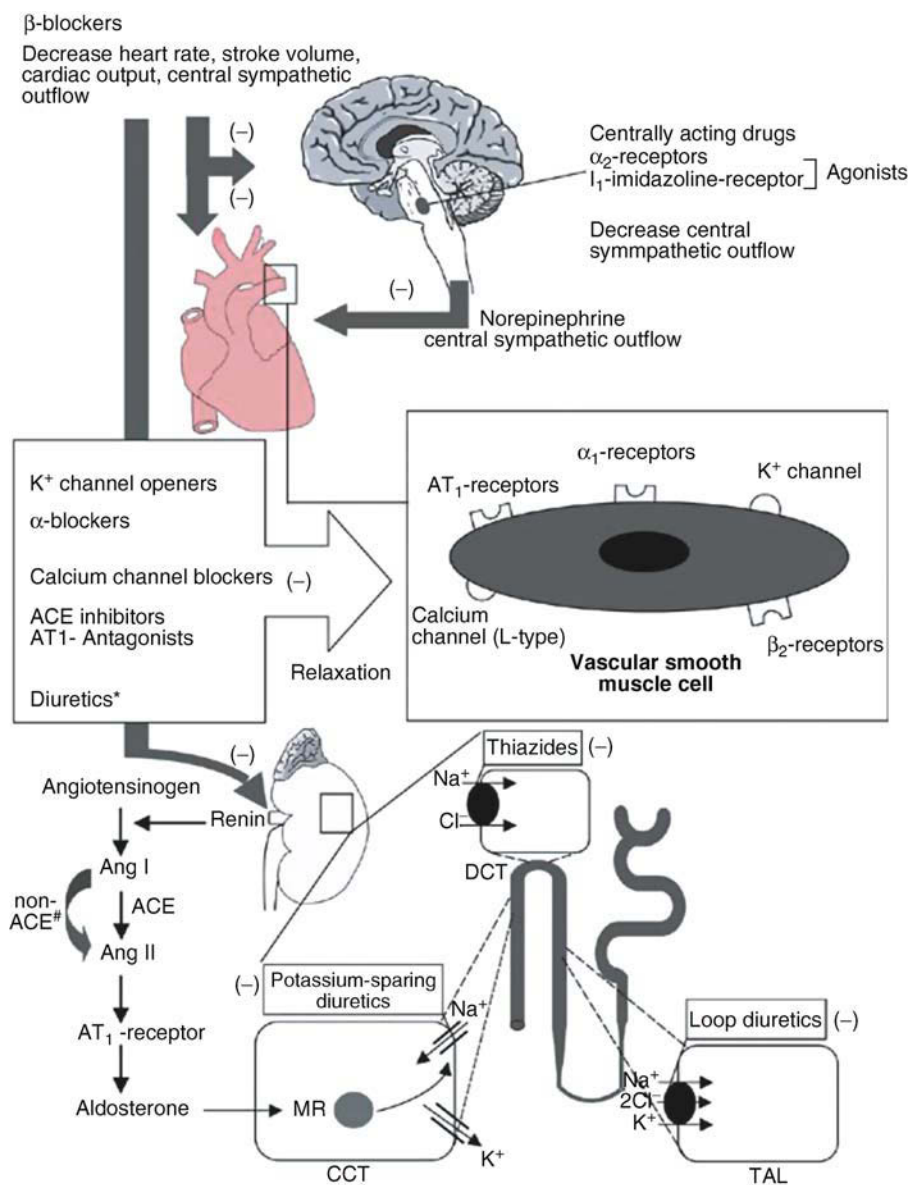
Definition

Reducing blood pressure by pharmacological means reduces cardiovascular morbidity and mortality rates. Benefits include protection from stroke, coronary events, heart failure, progression of renal disease, progression to more severe hypertension, and, most importantly mortality from all causes. Owing to the complexity of the pathogenesis of hypertension, antihypertensive drugs are directed against a variety of pharmacological targets in various cell types in different organs involved in blood pressure control.

Mechanism of Action

The fundamental mechanisms involved in blood pressure control have been outlined in chapter “Blood pressure control.” In addition to direct neuronal modulation of arterial pressure two neurohumoral systems, i.e., the sympathetic nervous system and the renin–angiotensin–aldosterone system (RAS) play a pivotal role in blood pressure control. Both systems are always either directly or indirectly affected by treatment with any antihypertensive drug. Antihypertensive agents can be categorized into seven different drug classes (Fig. 1) [1]. Centrally-acting antihypertensive drugs can be classified according to their relative affinities to α_2 - and imidazoline (I_1) receptors. Clonidine is considered as a mixed α_2 - and I_1 -agonist, whereas moxidine acts as a relative selective I_1 -agonist. Methyldopa is a selective α_2 -agonist. Administration of clonidine results in decreased cardiac output, a preservation of baroreflexes with a relative reduction in tendency of heart rate to rise, and little change in peripheral resistance. Methyldopa decreases sympathetic outflow predominantly to the α_1 -receptors of the arterioles, thereby reducing peripheral resistance with little (but some) effect on the heart. The baroreceptor arc is impaired because of the effect on arterioles [2]. Direct vasodilators such as minoxidil and hydralazine work by opening potassium channels in vascular smooth muscle cells in arterioles, which leads to K^+ efflux and hyperpolarization. Since the heart is not directly affected, direct vasodilators lead when used alone to reflex increases in heart rate and force of contraction; a significant neurohumoral activation of both the sympathetic nervous system and RAS occurs. Because of the activation of counterregulatory systems, the simultaneous use of β -blockers and diuretics is generally required [3]. Selective α_1 -blockers inhibit the action of norepinephrine (noradrenaline) at arteriolar receptors, thereby leading also to activation of counterregulatory systems [4]. Calcium channel blockers act primarily as inhibitors of vasoconstriction by blocking L-type calcium channels in vascular smooth muscle cells. However, there are two different main classes (dihydropyridines and nondihydropyridines), which work on different sites within the L-channel and hence produce different effects in the kidney, heart and vasculature. The nondihydropyridines

verapamil and diltiazem blunt increases in heart rate in response to exercise and have both negative inotropic and negative chronotropic (verapamil > diltiazem) effects; most dihydropyridines do not have major cardiodepressant effects because the long-acting agents slightly increase sympathetic nervous system tone, while this negative effect is even more pronounced with short-acting agents. In general, dihydropyridines lead to increases in heart rate and do not blunt the increase in heart rate response to exercise. Calcium channel blockers have a slight (transient) natriuretic effect [5]. The competitive inhibition of β -blockers on β -receptors results in numerous effects on functions that regulate blood pressure, including a reduction in cardiac output, a decrease in renin release, perhaps a decrease in both central sympathetic nervous outflow and peripheral resistance. The view that the primary effect is a reduction in cardiac output as a result from the blockade of cardiac β_1 -receptors with a subsequent reduction of heart rate and myocardial contractility has been questioned. Indeed, it seems that although cardiac output usually falls acutely and remains lower chronically, peripheral resistance on the other hand rises acutely but falls towards, if not to, normal with time. Thus, the hemodynamic hallmark of chronic established hypertension, which is an increased peripheral resistance, is also normalized by β -blockers. All currently available β -blockers antagonize cardiac β_1 -receptors competitively, but they vary in their degree of β_2 -receptor blockade in extra cardiac tissues. However, there seems to be little difference in antihypertensive efficacy among those that are more or less cardio- or β_1 -selective. Although the presence of intrinsic sympathomimetic activity (ISA) in some compounds such as pindolol and acebutolol could in theory translate into some beneficial effects, there is little convincing evidence that partial agonism confers significant clinical benefits. The newer compounds carvedilol and nebivolol produce additional vasodilator features that are attributable either to the additional blockade of α -receptors (carvedilol) or endothelial nitric oxide release (nebivolol). The mode of action of diuretics depends on their major site of action within the nephron (Fig. 1). These differences determine their relative efficacy as expressed in the maximal percentage of filtered sodium excreted. Sixty percent of the filtered sodium is reabsorbed in the proximal tubule of the nephron. Thirty percent is reabsorbed in the thick ascending limb of Henle by $\blacktriangleright Na^+/K^+/2Cl^-$ cotransport ($\blacktriangleright Na^+/K^+/2Cl^-$ cotransporter), which is inhibited by loop diuretics. Seven percent is reclaimed by Na^+/Cl^- cotransport in the distal convoluted tubule, which is inhibited by thiazide diuretics. The last 2% is reabsorbed via the $\blacktriangleright$ epithelial Na^+ channel (ENaC) in the cortical collecting duct, which is a target either directly (amiloride, triamterene) or indirectly via the mineralocorticoid receptor (spironolactone) for potassium-sparing agents (Fig. 1).



Antihypertensive Drugs. Figure 1 Site of action of different classes of antihypertensive drugs. Antihypertensive drugs are directed against a variety of pharmacological targets in various cell types in different organs involved in blood pressure control. The most important targets in the brain, heart, vasculature (vascular smooth muscle cells), and the kidney (nephron) are shown. *Some diuretics produce some direct vasodilation; # non-ACE, conversion of angiotensin I (Ang I) to angiotensin II (Ang II) may occur independent from ACE due to the activity of other enzymes in different tissues such as chymase in the heart; DCT, distal convoluted tubule; CCT, cortical collecting duct; TAL, thick ascending limb of the loop of Henle; (-), indicates inhibition. Modified according to reference 3.

Agents acting in the proximal tubule are seldom used to treat hypertension. Treatment is usually initiated with a thiazide-type diuretic. Chlorthalidone and indapamide are structurally different from thiazides but are functionally related. If renal function is severely impaired (i.e., serum creatinine above 2.5 mg/dl), a loop diuretic is needed. A potassium-sparing agent may be given with the diuretic to reduce the likelihood of hypokalemia.

By themselves, potassium-sparing agents are relatively weak antihypertensives. In general, there are four ways to reduce the activity of the RAS. The first way is the use of β -blockers to reduce renin release from the juxtaglomerular (JG). The second way, the direct inhibition of the activity of renin, although being actively investigated has not been successful in the clinical arena thus far. The third way is to inhibit the activity of the

► **angiotensin converting enzyme (ACE)**, which converts the inactive decapeptide angiotensin I to the potent octapeptide angiotensin II (Ang II), by agents referred to as ACE inhibitors. Thus, these agents inhibit the biosynthesis of Ang II and thereby decrease the availability of Ang II at both angiotensin type 1 (AT₁) and angiotensin type 2 (AT₂) receptors. The fourth way is to use a competitive and selective antagonist at the AT₁ receptor (i.e., AT₁ antagonists) and thereby to inhibit the classical effects mediated by Ang II such as vasoconstriction and aldosterone release. ACE inhibitors exhibit additional effects that are independent from RAS such as on kinins, since ACE is also a kininase. Although the clinical relevance is not fully understood, blood pressure effects mediated via inhibition of breakdown of bradykinin may contribute to the vasodilatory effects of ACE inhibitors. Some of the latter effects could be mediated via kinin stimulation of prostaglandin production. In addition to these effects on vascular tone, multiple other effects may contribute to the antihypertensive effects of ACE inhibitors. The blunting of the expected increase in sympathetic nervous activity typically seen after vasodilation is potentially of greater importance for the documented clinical benefits of ACE inhibitors. As a result, heart rate is not increased as is seen with direct vasodilators, α -blockers and less pronounced with dihydropyridine calcium channel blockers. The presence of the complete RAS within various tissues including the vasculature, kidney, heart and brain has been demonstrated and the activation of the RAS at the tissue level seems to play - beyond its role on blood pressure regulation - an important role for the manifestation and progression of hypertensive target organ damage in these organs. Our understanding of the molecular mechanisms by which Ang II contributes to both structural and functional changes, e.g., due to its growth factor capacity,

at the tissue level continues to expand. Consequently, the inhibition of tissue ACE may play an important role for the prevention and regression of hypertensive target organ damage that has been documented for these agents in experimental and clinical studies.

The major obvious difference between AT₁ antagonists and ACE inhibitors is the absence of an increase in kinins that may be responsible for some of the beneficial effects of ACE inhibitors and probably their side effects. Direct comparison between the two types of drugs show little differences in antihypertensive efficacy but cough, a common side effect seen with ACE inhibitors, is not provoked by AT₁ antagonists, although angioedema and agusia have also been reported for these newer agents.

Clinical Use (Including Side Effects)

Recent consensus committees, including the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI) and the World Health Organization-International Society of Hypertension (WHO-ISH) Guidelines Subcommittee, have modified traditional treatment recommendations in several important ways.

Criteria for initiation of drug treatment now take into consideration total cardiovascular risk rather than blood pressure alone, such that treatment is now recommended for persons whose blood pressure is in the normal range but still bear a heavy burden of cardiovascular risk factors. Thus, the role of simultaneous reduction of multiple cardiovascular risk factors in improving prognosis in hypertensive patients is stressed. In addition, more aggressive blood pressure goals are recommended for hypertensive patients with comorbid conditions such as diabetes mellitus or renal insufficiency.

Antihypertensive Drugs. Table 1 Common side effects of antihypertensive drugs

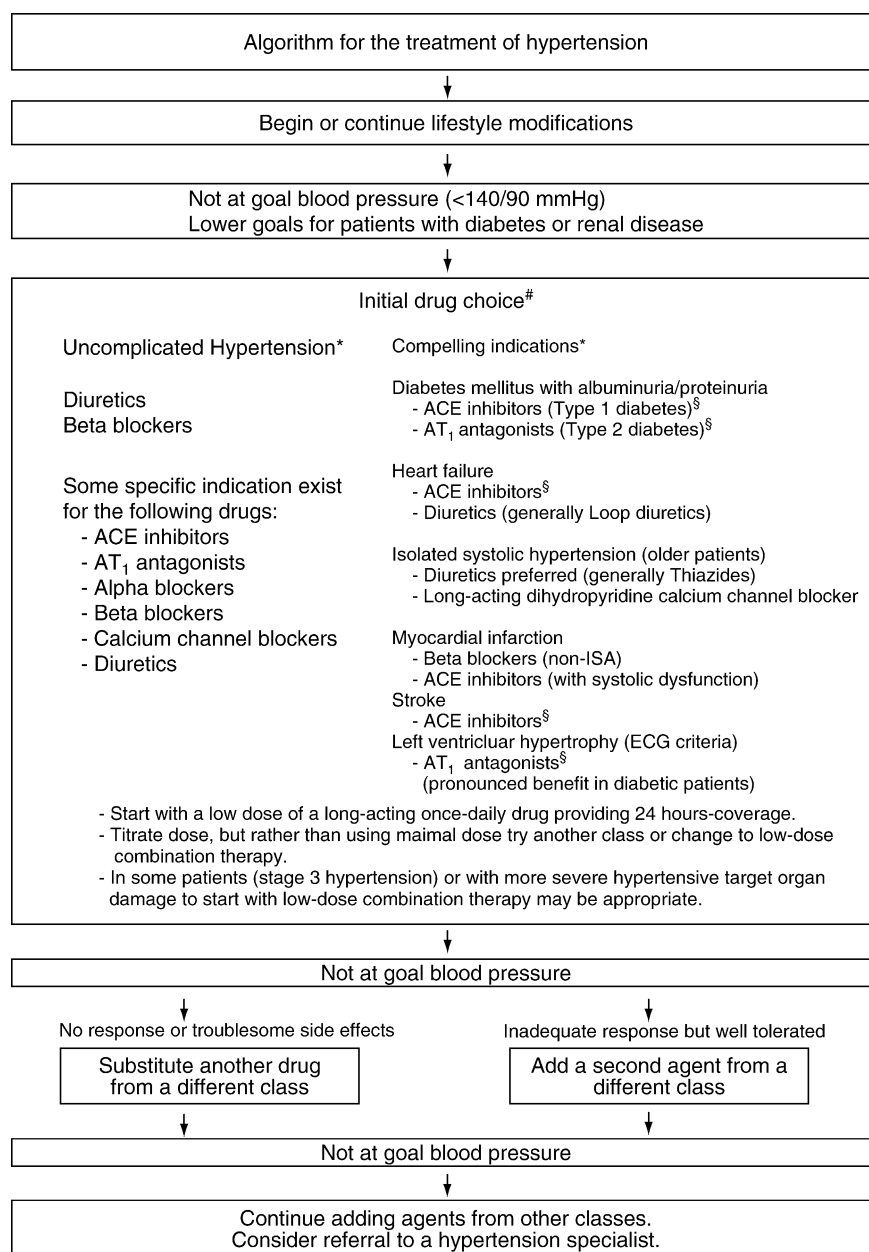
Class of drug		Side effects
ACE inhibitors		Cough, hyperkalemia, skin reactions
AT ₁ -antagonists		Hyperkalemia (less frequent compared with ACE inhibitors)
Calcium channel blockers	Dihydropyridine	Pedal edema, headache
	Nondihydropyridine	Constipation (verapamil); headache (diltiazem)
Diuretics		Frequent urination, hyperuricemia, hyperglycemia, hyperlipidemia
Centrally acting drugs	α 2-Receptor agonists	Sedation, dry mouth, rebound hypertension
	Imidazoline-receptor agonists	
Central neuronal blockers (reserpine)		Depression, sedation, nasal congestion
α -Blockers		Orthostatic hypotension, rapid drop of blood pressure after first dose, pedal edema, dizziness
β -Blockers		Fatigue, hyperglycemia, bronchospasm
Potassium channel openers		Hypertrichosis (minoxidil); lupus-like reactions and pedal edema (hydralazine)

Modified according to reference 3.

Finally, drug treatment in the elderly is of great importance and warrants special attention with regard to safety and tolerability, since systolic blood pressure is recognized as an important target for treatment, particularly in older persons. The benefits of antihypertensive treatment in the elderly and in patients with isolated systolic hypertension are greater than in younger persons.

As a consequence for drug treatment, an increasing number of patients will be treated with antihypertensive

compounds and the importance of tailoring the choice of antihypertensive drug treatment to the patient's individual profile of concomitant cardiovascular risk factors/comorbid conditions has to be emphasized. Moreover, it is reasonable to individualize antihypertensive treatment on the basis of each patient's personal needs with respect to tolerability, convenience and quality of life. Initiation of treatment with a drug that is expected to be well tolerated and therefore likely to be effective in lowering blood pressure over time is



Antihypertensive Drugs. Figure 2 Algorithm for the treatment of hypertension. # Unless contraindicated; *based on randomized controlled trials; § Evidence suggests that the beneficial effects of ACE inhibitors can be duplicated with AT₁ antagonists (and probably vice versa). Thus, ACE inhibitors could be substituted by AT₁ antagonists in the case of troublesome side effects, such as cough under treatment with ACE inhibitors. Modified according to reference 5.

prudent (common side effects are listed in Table 1). Long-acting agents are preferable because adherence to therapy and consistency of blood pressure control are superior when the drug is taken once a day. Low-dose, fixed-dose combination therapy can be used in place of monotherapy as initial treatment or as an alternative to adding a second agent of a different therapeutic class to unsuccessful monotherapy. The advantage of this approach is that low doses of drugs that act by different mechanisms may have additive or synergistic effects on blood pressure with minimal dose-dependent adverse effects. Giving the patient a single tablet provides an additional benefit. A case in point represents the well-established combination of an ACE inhibitor or AT1-antagonist with low-dose hydrochlorothiazide, which does not produce more side effects than placebo.

Many of the concepts of antihypertensive treatment put forward are adopted from the algorithms recommended by the JNC VI (Fig. 2). Treatment should always include lifestyle modifications. For the minority of hypertensive patients without comorbid conditions, target organ damage, or concomitant cardiovascular disease, the JNC VI recommends starting drug therapy with a diuretic (i.e., thiazides) or β -blocker because these agents had been proven to lower morbidity and mortality compared with placebo in randomized controlled trials. Secondly, they are less costly than newer classes of drugs. Therefore, the era of placebo-controlled trials is past and any new agents can only be compared against the gold standard of diuretics and β -blockers. Overall, these early trials with diuretics and β -blockers established a greater reduction of risk related to stroke (–40%) than the risk related to coronary heart disease (–14%). While diuretics are more effective in preventing stroke than β -blockers the opposite holds true for cardiac risk. A reduction in cardiovascular risk has also been documented for the ACE-inhibitor captopril and in elderly patients with isolated systolic hypertension for the dihydropyridine calcium channel blocker (nitrendipine). However, outcome trials comparing two anti-hypertensives require large groups of patients, because the risk in patients with mild-to-moderate essential hypertension is low, and intervention trials are usually limited to duration of 5 years. Therefore, differences between drug classes have been documented when patients with higher absolute cardiovascular risk and/or comorbid conditions were studied. Compelling indications that have been established in randomized controlled trials are summarized in Fig. 2.

- ▶ ACE Inhibitors
- ▶ Blood Pressure Control
- ▶ Ca^{2+} Channel Blockers
- ▶ Diuretics
- ▶ Renin–Angiotensin–Aldosterone System

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Anti-inflammatory Drugs

▶ Non-steroidal Anti-inflammatory Drugs

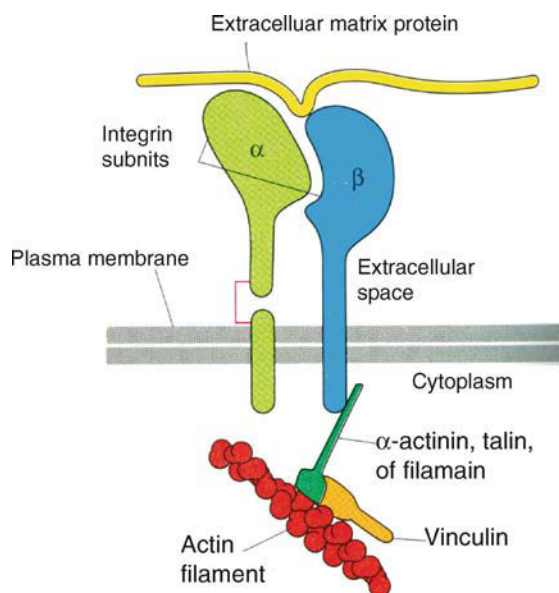
Anti-integrins

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Definition

Integrins are a widely expressed family of cell adhesion receptors via which cells attach to extracellular matrices either to each other or to different cells. All integrins are composed of $\alpha\beta$ heterodimeric units (Fig. 1), expressed on a wide variety of cells, and most cells express several integrins. The interaction of integrins with the cytoskeleton and extracellular matrix appears to require the presence of both subunits. The binding of integrins to their ligands is cation-dependent. Integrins appear to recognize specific amino acid sequences in their ligands. The best studied is the RGD sequence found within a number of ▶matrix proteins including fibrinogen, vitronectin, fibronectin, thrombospondin, osteopontin and VWF (Fig. 1). However, other integrins bind to ligands via non-RGD binding domain such as the $\alpha 4\beta 1$ integrin receptors that bind and recognize the LDV sequence within the CS-1 region of fibronectin. There are at least 8 known β subunits and 14 α subunits [1]. Although the association of the different β and α subunits



Anti-integrins. Figure 1

could in theory result in more than 100 integrins, the actual diversity is restricted.

Basic Characteristics

Integrin adhesion receptors contain an extracellular face that engages adhesive ligands and a cytoplasmic face that engages with intracellular proteins. The interactions between the cell adhesion molecules and extracellular matrix proteins are critical for cell adhesion and for anchorage-dependent signaling reactions in normal and pathological states. For example, platelet activation induces a conformational change in integrin $\alpha\text{IIb}\beta_3$, thereby converting it into a high affinity fibrinogen receptor. Fibrinogen binding then triggers a cascade of protein tyrosine kinases, phosphatases and recruitment of numerous other signaling molecules into F-actin-rich cytoskeletal assemblies in proximity to the cytoplasmic tails of αIIb and β_3 [2]. These dynamics appear to influence platelet functions by coordinating signals emanating from integrins and G protein-linked receptors. Studies of integrin mutations confirm that the cytoplasmic tails of $\alpha\text{IIb}\beta_3$ are involved in integrin signaling presumably through direct interactions with cytoskeletal and signaling molecules [2]. Blockade of fibrinogen binding to the extracellular face of $\alpha\text{IIb}\beta_3$ has been shown to be an effective way to prevent arterial $\blacktriangleright$ thrombosis after coronary angioplasty in myocardial infarction and unstable angina patients [2].

Mechanism of Action and Clinical Use Pathophysiology and Therapeutic Potential

The role of integrins has been found in various pathological processes, including $\blacktriangleright$ angiogenesis,

thrombosis, apoptosis, cell migration and proliferation. These processes lead to both acute and chronic diseases such as ocular diseases, metastasis, unstable angina, myocardial infarction, stroke, $\blacktriangleright$ osteoporosis, and a wide range of inflammatory diseases, vascular remodeling and neurodegenerative disorders. A breakthrough in this field is evident from the role of the platelet $\alpha\text{IIb}\beta_3$ integrin in the prevention, treatment and diagnosis of various thromboembolic disorders. Additionally, significant progress in the development of leukocyte $\alpha_4\beta_1$ antagonists for various inflammatory indications and α_v integrin antagonists for angiogenesis and vascular-related disorders has been achieved.

β_1 Integrins $\alpha_4\beta_1$ Integrin

The largest numbers of integrins are members of the β_1 integrins, also known as the very late antigen (VLA) subfamily because of its late appearance after activation. There are at least seven receptors characterized from this subfamily, each with different ligand specificity. Among the most studied include the $\alpha_4\beta_1$ and $\alpha_5\beta_1$ receptors. The leukocyte integrin $\alpha_4\beta_1$ is a cell adhesion receptor that is predominantly expressed on lymphocytes, monocytes and eosinophils.

Potent and Selective Small Molecule Antagonists of $\alpha_4\beta_1$ Integrins

The $\alpha_4\beta_1$ integrins are heterodimeric cell surface molecules central to leukocyte-cell and leukocyte-matrix adhesive interactions. The integrin $\alpha_4\beta_1$, expressed on all leukocytes except neutrophil, interacts with the immunoglobulin superfamily member VCAM-1 and with an alternately spliced form of fibronectin. Additionally, the integrin $\alpha_4\beta_7$ is also restricted to leukocytes and can bind not only to VCAM1 and fibronectin, but also to MAdCAM the mucosal addressin or homing receptor, which contains Ig-like domains related to VCAM-1. *In vivo* studies with $\alpha_4\beta_1$ monoclonal antibodies in several species demonstrate that the interactions between these integrins and their ligands play a key role in immune and $\blacktriangleright$ inflammatory disorders [3] and selected ones are in clinical trials.

$\alpha_5\beta_1$ Integrin in Angiogenesis

In contrast to collagen, expression of the extracellular matrix protein fibronectin in provisional vascular matrices precedes permanent collagen expression and provides signals to vascular cells and fibroblasts during blood clotting and wound healing, atherosclerosis and hypertension. Fibronectin expression is also upregulated on blood vessels in granulation tissues during wound healing. These observations suggest a possible role for this isoform of fibronectin in angiogenesis. Evidence was recently provided that both fibronectin and its receptor integrin $\alpha_5\beta_1$ directly

regulate angiogenesis [4]. Thus, integrin antagonist for $\alpha 5 \beta 1$ integrin might be a useful target for the inhibition of angiogenesis associated with human tumor growth; neovascular related ocular and inflammatory diseases.

$\alpha 5 \beta 1$ Integrin and Bacterial Invasion

Recent studies suggested a key role for $\alpha 5 \beta 1$ integrin in certain bacterial invasion of human host cells leading to antibiotic resistance [5].

$\beta 3$ Integrins

Intravenous and Oral Platelet IIb/ $\beta 3$ Receptor

Antagonists: Potential Clinical Utilities

There is an urgent need for more efficacious antithrombotic drugs superior to aspirin or ticlopidine for the prevention and treatment of various cardiovascular and cerebrovascular thromboembolic disorders. The realization that the platelet integrin $\alpha IIb \beta 3$ is the final common pathway for platelet aggregation regardless of the mechanism of action prompted the development of several small molecule $\alpha IIb / \beta 3$ receptor antagonists for intravenous and/or oral antithrombotic utilities. Platelet $\alpha IIb / \beta 3$ receptor blockade represents a very promising therapeutic and diagnostic strategy of thromboembolic disorders. Clinical experiences (efficacy/safety) gained with injectable but not oral $\alpha IIb \beta 3$ antagonists (Abciximab, Eptifibatide, Aggrastat) elucidate the safety and efficacy of this mechanism in combination with other antiplatelet and anticoagulant therapies.

Orally Active GPIIb/IIIa Antagonists

A high level of platelet antagonism has been required when GPIIb/IIIa antagonists have been employed for acute therapy of coronary arterial diseases using intravenous GPIIb/IIIa antagonists with heparin and aspirin. Interaction with aspirin and other antiplatelet and anticoagulant drugs lead to shifts in the dose-response curves for both efficacy and unwanted side effects, such as increased bleeding time. More recently, all oral GPIIb/IIIa antagonists with or without aspirin but not with anticoagulant were withdrawn because of a disappointing outcome (no clinical benefit or increased thrombotic events). This raises a lot of serious questions with regard to the potential of oral GPIIb/IIIa antagonists as compared to the well-documented success of intravenous GPIIb/IIIa antagonists.

GPIIb/IIIa Integrin Receptor Antagonists in the Rapid Diagnosis of Thromboembolic Events

The role of the platelet integrin GPIIb/IIIa receptor and its potential utility as a radio-diagnostic agent in the rapid detection of thromboembolic events has been demonstrated [6]. This approach may be useful for the noninvasive diagnosis of various thromboembolic disorders.

Integrin $\alpha \nu \beta 3$ Antagonists Promote Tumor Regression by Inducing Apoptosis of Angiogenic Blood Vessels

Antagonists of integrin $\alpha \nu \beta 3$ inhibit the growth of new blood vessels into tumors cultured on the chick chorioallantoic membrane without affecting adjacent blood vessels, and also induce tumor regression [7]. Antagonists of $\alpha \nu \beta 3$ also inhibit angiogenesis in various ocular models of retinal neovascularization [7].

Integrin $\alpha \nu \beta 3$ in Restenosis

The calcification of atherosclerotic plaques may be induced by osteopontin expression, since osteopontin is a protein with a well-characterized role in bone formation and calcification. Vascular smooth muscle cell migration on osteopontin is dependent on the integrin $\alpha \nu \beta 3$ and antagonists of $\alpha \nu \beta 3$ prevent both smooth muscle cell migration and restenosis in some animal model [8].

Integrin $\alpha \nu \beta 3$ Antagonists Versus Anti- $\alpha \nu \beta 3$ and $\alpha \nu \beta 5$

Since the recognition of at least two $\alpha \nu$ integrin pathways for cytokine-mediated angiogenesis, $\alpha \nu \beta 3$ and $\alpha \nu \beta 5$ antagonists may be more effective in certain indications as compared to a specific anti- $\alpha \nu \beta 3$. However, further work is needed to document this notion.

Potential Role of $\alpha \nu \beta 3$ Antagonists in Osteoporosis

RGD analogs have been shown to inhibit the attachment of osteoclasts to bone matrix and to reduce bone resorptive activity *in vitro*. The cell surface integrin, $\alpha \nu \beta 3$, appears to play a role in this process. RGD analogs may represent a new approach to modulating osteoclast-mediated bone resorption and may be useful in the treatment of osteoporosis [9].

Integrins $\alpha \nu \beta 3$ Ligands

Therapeutics: A number of potent small molecule antagonists for $\alpha \nu \beta 3$ integrin are under preclinical investigations for various angiogenesis or vascular-mediated disorders [10].

Site directed delivery: This approach of conjugating $\alpha \nu \beta 3$ integrin ligand with a chemotherapeutic agent for optimal efficacy and safety in cancer is under investigation. Earlier work demonstrated the validity of this concept [10].

Diagnostics: Imaging metastatic cancer using technetium-99m labeled RGD-containing synthetic peptide has been demonstrated. Additionally, detection of tumor angiogenesis *in vivo* by $\alpha \nu \beta 3$ -targeted magnetic resonance imaging (MRI) was demonstrated [10].

- Angiogenesis and Vascular Morphogenesis
- Antiplatelet Drugs

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Antimalarial Drugs

Antiprotozoal Drugs.

Antimetabolic Agents

► Antimetabolites

Antimetabolites

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Synonyms

Anticancer antimetabolites; Antimetabolic agents; Antineoplastic antimetabolites

Definition

Antimetabolites compete with and possibly oust naturally occurring metabolites required for normal biochemical reactions and lead either to the synthesis of malfunctioning macromolecules and/or blockade of necessary intermediate or final metabolic products that are vital to cell survival. Both processes interfere with DNA synthesis and therefore antimetabolites can be used in cancer treatment, as they inhibit cell division and the growth of tumors.

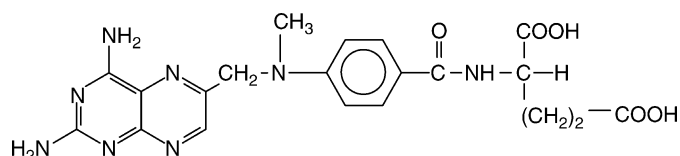
Mechanism of Action

A broad description of antimetabolites would include compounds with structural similarity to precursors of purines or pyrimidines or agents that interfere with purine and pyrimidine synthesis. Antimetabolites can cause either DNA damage indirectly through misincorporation into DNA followed by abnormal timing or progression through DNA synthesis, or altered function of enzymes involved in pyrimidine and purine synthesis. All antimetabolites tend to exert greatest cytotoxicity in the S-phase of cells and the duration of exposure is of great importance. The antimetabolite activity is rather unselective and affects all fast proliferating cells. In contrast to alkylating agents, second malignancies are not associated with their use. The following is a list of commonly used antimetabolites [1].

1. Folate antagonists
 - Methotrexate
 - Ralitrexed
 - Pemetrexed (alimta)
2. Purine antagonists
 - Mercaptopurine (6-MP)
 - Thioguanine (6-TG)
 - Pentostatine
 - Fludarabine phosphate (fludara, F-ara-A)
3. Pyrimidine antagonists
 - Fluorouracil (5-FU)
 - Cytosine arabinoside (cytarabine, Ara C)
 - Gemcitabine (gemzar)

Some newly introduced pyrimidine analogs (e.g., azacitidine and decitabine) differ in their mechanism of action to such a degree from the other antimetabolites that they are subgrouped under the heading DNA de- or ►hypomethylating agents (see below).

Methotrexate (MTX, chemical structure shown in Fig. 1.) competitively inhibits the dehydrofolate reductase, an enzyme that plays an essential role in purine synthesis. The dehydrofolate reductase regenerates reduced folates when thymidine monophosphate is formed from deoxyuridine monophosphate. Without reduced folates cells are unable to synthesize thymine. Administration of *N*-5 tetrahydrofolate or *N*-5 formyl-tetrahydrofolate (folinic acid) can bypass this block and rescue cells from methotrexate activity by serving as antidote.



Antimetabolites. Figure 1 Chemical structure of methotrexate.

MTX and naturally occurring reduced folates are transported into cells by the folate carrier which has a higher affinity to MTX than to the natural folates. Inside of cells, MTX will be polyglutamated and thus enter a deep compartment from which it can be released again only slowly. High extracellular concentrations can bypass this carrier by passive diffusion. MTX is well absorbed orally and at usual dosages half of its level is bound to plasma proteins. After intravenous application the decay is triphasic: distribution phase; initial elimination and a prolonged elimination, the latter corresponding to the slow release from the intracellular polyglutamate compartment.

Clinical Use (including Side-Effects)

This scenario is the background for designing high dose methotrexate regimens with leucovorin rescue of normal hematopoietic and mucosa cells as part of curative therapy schedules for osteosarcoma and hematological neoplasias of children and adults. Methotrexate is cleared from the plasma by the kidney by both glomerular filtration and tubular secretion. Therefore the toxicity of this drug is augmented by renal dysfunction.

MTX is part of curative therapeutic schedules for acute lymphoblastic leukemias (ALL), Burkitt's lymphoma, and choriocarcinoma. It was also used in adjuvant therapy of breast cancer. High dose MTX with leucovorin rescue can induce about 30% remissions in patients with metastatic osteogenic sarcoma. MTX is one of the few antineoplastic drugs that can be safely administered intrathecally for the treatment of meningeal metastases and leukemic infiltrations (routine prophylaxis in ALL). In addition, MTX can be used as an immunosuppressive agent for the treatment of severe rheumatoid arthritis and psoriasis.

Myelosuppression is the major dose-limiting side effect. Gastrointestinal toxicity may appear as ulcerative mucositis and diarrhea. Nausea and vomiting, alopecia, and skin inflammation are common following high-dose MTX treatment. Renal toxicity has major impact for high-dose regimens with MTX and the drug should not be used in patients with renal injury. ▶**Intrathecal application** may be associated with mild arachnoiditis or severe and progressive myelo- or encephalopathy. Chronic low dose MTX may cause cirrhosis of the liver. MTX is a potent ▶**teratogen** and abortifacient.

Ralitrexed is a folate analog with greater selectivity. It easily crosses the cell membrane and undergoes polyglutamation. Within tissues, ralitrexed may be stored up to 29 days. It directly inhibits thymidylate synthase, the key enzyme for synthesizing thymidine triphosphate (TTP). The drug has been described to induce apoptosis in tumor cells. Ralitrexed is used for the treatment of colon carcinomas.

Pemetrexed is an antifolate ▶**antineoplastic agent** that exerts its action by disrupting folate-dependent metabolic processes essential for cell replication. Pemetrexed disodium heptahydrate has the chemical name L-Glutamic acid, *N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo [2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-, disodium salt, heptahydrate. Pemetrexed is an antifolate containing the pyrrolopyrimidine-based nucleus that exerts its antineoplastic activity by disrupting folate-dependent metabolic processes essential for cell replication. In vitro studies have shown that pemetrexed also inhibits thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycylamide ribonucleotide formyltransferase (GARFT), which are all folate-dependent enzymes involved in the *de novo* biosynthesis of thymidine and purine nucleotides. Pemetrexed is transported into cells by both the reduced ▶**folate** carrier and the membrane folate binding protein transport systems. Once in the cell, pemetrexed is converted to polyglutamate forms by the enzyme folylpolyglutamate synthetase. The polyglutamate forms are retained intracellular and are inhibitors of TS and GARFT. Polyglutamation is a time- and concentration-dependent process that occurs in tumor cells and, to a lesser extent, in normal tissues. Polyglutamated metabolites have an increased intracellular half-life resulting in prolonged drug action in malignant cells. Preclinical studies have shown that pemetrexed inhibits the *in vitro* growth of mesothelioma cell lines, an effect which was synergistically increased in combination with cisplatin.

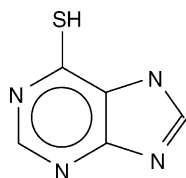
The recommended dose of pemetrexed is 500 mg/m² administered as an intravenous infusion over 10 min on Day 1 of each 21-day cycle. Pemetrexed is not metabolized to an appreciable extent and is primarily eliminated in the urine, with 70-90% of the dose recovered unchanged within the first 24 h following administration. Pemetrexed has a steady-state volume of distribution of 16.1 L. Pemetrexed is highly bound (approximately 81%) to plasma proteins. Binding is not affected by the degree of renal impairment. Plasma

clearance of pemetrexed in the presence of cisplatin decreases as renal function decreases, with increase in systemic exposure. Patients with creatinine clearances of 45, 50, and 80 mL/min had 65%, 54%, and 13% increases, respectively in pemetrexed total systemic exposure (AUC) compared to patients with creatinine clearance of 100 mL/min. Pemetrexed in combination with cisplatin is used for the treatment of patients with malignant pleural mesothelioma. As a single agent it is used for treating patients with locally advanced or metastatic non-small cell lung cancer after prior ►chemotherapy. The main dose-limiting side effect is myelosuppression. Skin rashes and neurotoxic reactions have been recorded, too.

Mercaptopurine (6-MP, Fig. 2) is an analog of hypoxanthine and was one of the first agents shown to be active against acute leukemias. It is part of maintenance therapy for ALL. Mercaptopurine has to be activated by hypoxanthine guanine-phosphoribosyl-transferase (HGPRTase) to 6-MP monophosphate. This metabolite inhibits the synthesis of adenine and guanine at the initial aminotransferase step and suppresses the conversion of inosinic acid to adenylylate and guanylate. Some part of the drug is also incorporated into DNA in the form of thioguanine. Resistance to 6-MP may be associated with decreased drug activation by HGPRTase or increased inactivation by the alkaline phosphatase. The drug is slightly bound (20%) to plasma proteins and does not cross the blood-brain barrier. Xanthine oxidase is the primary enzyme responsible for its metabolic inactivation.

The plasma half-life of 6-MP after intravenous bolus injection is 21 min in children and is twofold greater in adults. After oral intake peak levels are attained within 2 h. 6-MP is used for the treatment of ALL and has shown certain activity in chronic myelogenous leukemia. The major side effects involve myelosuppression, nausea, vomiting, and hepatic injury.

Thioguanine (6-thioguanine, 6-TG; Fig. 3) is an analog of guanine in which a hydroxyl group has been substituted by a sulfhydryl group in position 6 of the purine ring. The mode of action involves two mechanisms: incorporation into DNA or RNA; and feedback inhibition of purine synthesis. Similarly to 6-MP, 6-TG undergoes initial activation by the enzyme HGPRTase. The corresponding monophosphate can be eventually



Antimetabolites. Figure 2 Chemical structure of mercaptopurine.

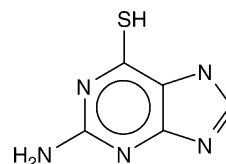
converted to deoxy-6-thioguanosine-triphosphate (dTGTP) and subsequently incorporated into DNA. Resistance to thioguanine has been correlated with decreased activity of HGPRTase and to inactivation by alkaline phosphatase. After oral intake the drug is slowly absorbed and peak levels of metabolites occur only after 6–8 h. Within the first 24 h a part (up to 46%) of the dose administered is excreted via the urine.

Thioguanine is used primarily as part of induction chemotherapy regimens for acute myelogenous leukemia (AML).

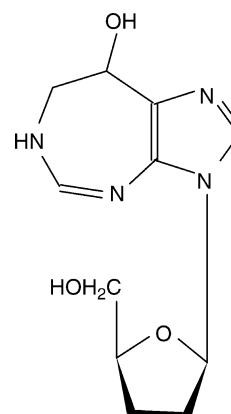
The most common adverse effects are myelosuppression, with leukopenia and thrombocytopenia appearing 7–10 days after treatment, as well as mild nausea. Liver toxicity with jaundice has been reported in rare cases.

Pentostatin (deoxycoryformycin; Fig. 4) is a purine isolated from cultures of *Streptomyces antibioticus*. Its mode of action involves inhibition of adenosine deaminase, which plays a key role in purine salvage pathways and DNA synthesis. As a consequence, deoxyadenosine triphosphate (dATP) is accumulated, which is highly toxic to lymphocytes. This is associated with augmented susceptibility to apoptosis, particularly in T cells.

Pentostatin is effective in the treatment of hairy cell leukemia, producing 80–90% remissions (with a complete remission rate of more than 50%). The common side effects of pentostatin include myelosuppression, nausea, and skin rashes. Renal failure,



Antimetabolites. Figure 3 Chemical structure of thioguanine.



Antimetabolites. Figure 4 Chemical structure of pentostatin (nipent).

immunosuppression and CNS dysfunction have also been observed.

Cladribine (2-Chlordeoxyadenosine) is a synthetic purine nucleoside that is converted to an active cytotoxic metabolite by the deoxycytidine kinase. The drug is relatively selective for both normal and malignant lymphoid cells.

Cladribine is highly active against hairy cell leukemia (complete remissions were achieved in more than 60% of patients receiving a single 7-day course). Activity has been recorded in other low-grade lymphoid malignancies. The major side effect is myelosuppression.

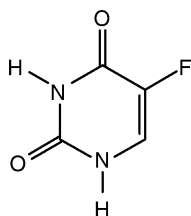
Fludarabine is a fluorinated purine analog of the antiviral drug vidarabine. The active metabolite, 2-fluoro-adenosine-arabinoside triphosphate (F-ara-A), inhibits various enzymes involved in DNA synthesis such as DNA-polymerase- α , ribonucleotide reductase, and DNA primase. F-ara-A is incorporated into DNA and can cause delayed cytotoxicity even in cells with low growth fraction, e.g., CLL and follicular B cell lymphoma. Unlike typical antimetabolites, it is toxic to nonproliferating cells of lymphoid origin. The drug is highly active against chronic lymphocytic leukemia (CLL) with approximately 40% of patients achieving remission after previous therapy with alkylating agents. Therapeutic responses can also be seen in low-grade lymphomas. Dose-limiting side effect is myelosuppression that contributes to fevers and infections in half of treated patients. Occasional neurotoxicity (agitation, confusion, visual disturbances) has been noted at higher doses. Recently, an oral drug formulation has been developed.

Fluorouracil (5-fluorouracil, 5-FU, [Fig. 5](#)) represents an early example of "rational" drug design in that it originated from the observation that tumor cells, especially from gut, incorporate radiolabeled uracil more efficiently into DNA than normal cells. 5-FU is a fluorinated pyrimidine analog that must be activated metabolically. In the cells 5-FU is converted to 5-fluoro-2'-deoxyuridine-monophosphate (FdUMP). This metabolite inhibits thymidilate synthase which catalyses the conversion of uridylylate (dUMP) to thymidilate (dTMP) whereby methylenetetrahydrofolate plays the role of the carbon-donating cofactor. The reduced folate cofactor occupies an allosteric site of

thymidilate synthase, which allows for the covalent binding of 5-FdUMP to the active site of the enzyme. In addition, misincorporation can induce single strand breaks, and RNA can aberrantly incorporate FdUMP. Leucovorin augments the activity of 5-FU by promoting formation of the ternary covalent complex consisting of 5-FU, the reduced folate, and thymidilate synthase. The drug is selectively toxic to proliferating rather than nonproliferating cells and is active in both the G1- and S-phases of the cell cycle. 5-FU is metabolized by dihydropyrimidine dehydrogenase and therefore deficiency of this enzyme can lead to excessive toxicity from 5-FU.

Capecitabine is a special prodrug of 5-FU. It is readily absorbed from the gastrointestinal tract. In the liver, a carboxylesterase hydrolyzes much of the compound to 5'-deoxy-5-fluorocytidine (5'-DFCR). Cytidine deaminase, an enzyme found in most tissues, including tumors, subsequently converts 5'-DFCR to 5'-deoxy-5-fluorouridine (5'-DFUR). Finally, the enzyme thymidine phosphorylase (dThdPase or TP), hydrolyzes 5'-DFUR to the active drug 5-FU. Both normal and tumor cells metabolize 5-FU to 5-fluoro-2'-deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). These metabolites cause cell injury by two different mechanisms. First, FdUMP and the folate cofactor N5-10-methylene-tetrahydrofolate bind to thymidilate synthase (TS) to form a covalently bound ternary complex. This binding inhibits the formation of thymidilate from 2'-deoxyuridylylate. Thymidilate is the necessary precursor of thymidine triphosphate, which is essential for the synthesis of DNA, so that a deficiency of this compound can inhibit cell division. Second, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of uridine triphosphate (UTP) during the synthesis of RNA. This metabolic error can interfere with RNA processing and protein synthesis. The addition of leucovorin to capecitabine is not recommended since there was no apparent advantage in response rate; however, toxicity was increased.

Capecitabine is used for the treatment of colorectal and breast cancers. It is contraindicated in patients with known hypersensitivity to capecitabine or any of its components or to 5-fluorouracil and in patients with known dihydropyrimidine dehydrogenase (DPD) deficiency. The use of capecitabine is restricted in patients with severe renal impairment. The drug can induce diarrhea, sometimes severe. Other side effects include anemia, hand-foot syndrome, hyperbilirubinemia, nausea, stomatitis, pyrexia, edema, constipation, dyspnea, neutropenia, back pain, and headache. Cardiotoxicity has been observed with capecitabine. A clinically important drug interaction between capecitabine and warfarin has been demonstrated. Care should be exercised when the drug is co-administered with CYP2X9 substrates.



Antimetabolites. Figure 5 Chemical structure of 5-fluorouracil.

Cytarabine (cytosine arabinoside, ara C, Fig. 6) is an analog of the pyrimidine nucleosides. It is one of the most potent agents available for treating acute myeloid leukemia. The drug must first be activated by pyrimidine nucleoside kinase to the triphosphate nucleotide ara-cytosine triphosphate (ara-CTP). The susceptibility of tumor cells to cytarabine is thought to be a reflection of their ability to activate the drug more rapidly by kinases than to inactivate it by deaminases. Cytarabine is incorporated into DNA and kills cells during the S-phase of the cycle by competitively inhibiting the DNA polymerase.

The drug is metabolized rapidly in the liver, kidney, intestinal mucosa, and even red blood cells. Therefore it has a plasma half-life of only 10 min after bolus intravenous application. The major metabolite, uracil arabinoside (ara-U), can be detected in the blood shortly after cytarabine administration. About 80% of the dose is excreted in the urine within 24 h, with less than 10% appearing as cytarabine; the remainder is ara-U. After continuous infusion, cytarabine levels in the liquor (cerebro-spinal fluid) approach 40% of that in plasma. Continuous infusion schedules allow maximal efficiency, with uptake peaks of 5-7 μM . It can be administered intrathecally as an alternative to methotrexate.

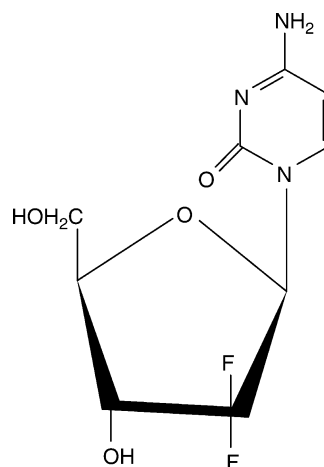
Cytarabine is used in the chemotherapy of acute myelogenous leukemia, usually in combination with anthracyclines, thioguanine, or both. It is less useful in acute lymphoblastic leukemia and lymphomas and has marginal activity against other tumors. Myelosuppression is a major toxicity, as is severe bone marrow hypoplasia; nausea and mucositis may also occur.

Gemcitabine (Fig. 7) is a cytosine derivative that is very similar to ara C. It undergoes metabolic activation to difluorodeoxycytidine triphosphate, which interferes

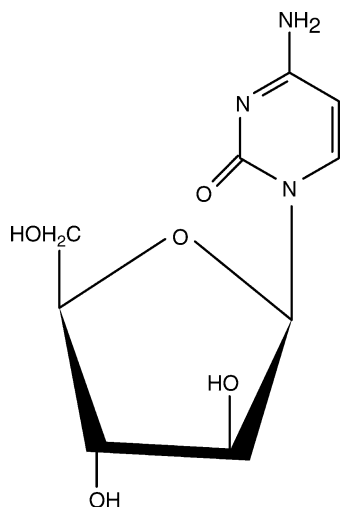
with DNA synthesis and repair. Gemcitabine causes the so called masked termination of DNA elongation. After incorporation into the DNA strand a normal nucleotide is further added. Therefore, DNA repair enzymes can not recognize and reconstruct the damaged DNA. In contrast to ara C, gemcitabine appears to have useful activity in a variety of solid tumors, with limited nonmyelosuppressive toxicities. Gemcitabine is a well known inducer of apoptosis at micro- to nanomolar concentrations. It is administered by intravenous infusion and has pharmacokinetics similar to ara C.

The usual dose consists of 1000 mg/m^2 i.v. It is the most active single agent for treating pancreatic cancer, and it is used as a first-line treatment for both pancreatic and small cell lung cancers. The dose-limiting toxicity is bone marrow suppression.

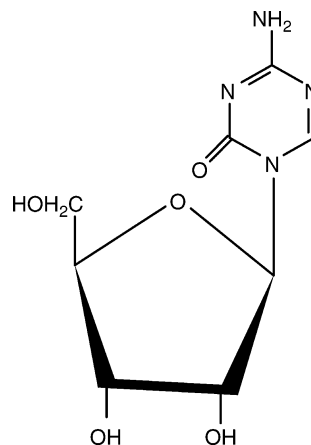
Azacitidine (5-aza-cytidine, Fig. 8) is a pyrimidine nucleoside analog of cytidine. The structural formula is



Antimetabolites. Figure 7 Chemical structure of gemcitabine (gemzar).



Antimetabolites. Figure 6 Chemical structure of cytarabine (ara C).



Antimetabolites. Figure 8 Chemical structure of Azacitidine (4-amino-1- β -D-ribofuranosyl-s-triazin-2 (1H)-one).

shown in Fig. 8. Azacitidine is believed to exert its antineoplastic effects by causing hypomethylation of DNA and direct cytotoxicity on abnormal hematopoietic cells in the bone marrow. The concentration of azacitidine required for maximum inhibition of DNA methylation in vitro does not cause major suppression of DNA synthesis. Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation. The cytotoxic effects of azacitidine cause the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms. Nonproliferating cells are relatively insensitive to azacitidine.

The pharmacokinetics of azacitidine shows that it is rapidly absorbed after s.c. administration; with the peak plasma concentration occurring after 0.5 h. The bioavailability of s.c. azacitidine relative to i.v. azacitidine is approximately 89%. Urinary excretion is the primary route of elimination of azacitidine and its metabolites. The mean elimination half-lives are about 4 h, regardless of i.v. or s.c. administration.

In vitro studies in human liver fractions indicated that azacitidine may be metabolized by the liver. Azacitidine and its metabolites are known to be substantially excreted by the kidney, and the risk of toxic reactions to this drug may be greater in patients with impaired renal function.

Azacitidine is used for treating patients with some myelodysplastic syndrome subtypes and chronic myelomonocytic leukemia. The most commonly occurring adverse reactions include nausea, anemia, thrombocytopenia, vomiting, pyrexia, leucopenia, diarrhea, fatigue, neutropenia, and ecchymosis.

Azacitidine is contraindicated in patients with a known hypersensitivity to azacitidine or mannitol as well as in patients with advanced ▶malignant hepatic tumors.

Decitabine (5-aza-deoxycytosine) is an analog of the nucleoside 2'-deoxycytidine. It is believed to exert its antineoplastic effects after phosphorylation and direct incorporation into DNA and by inhibition of the enzyme DNA methyltransferase, causing hypomethylation of DNA and cellular differentiation or apoptosis. DNA hypomethylation is achieved at concentrations below those required to significantly inhibit DNA synthesis, which may promote restoration of function to genes associated with control of cellular differentiation and proliferation. Cytotoxicity in rapidly dividing cells may also result from covalent adducts between DNA methyltransferase and decitabine.

Decitabine is specifically indicated for the treatment of multiple types of myelodysplastic syndromes and chronic myelomonocytic leukemia. As anticipated, use of decitabine is associated with bone marrow suppression including neutropenia and thrombocytopenia which are the most frequently observed serious adverse effects.

- ▶Antineoplastic Agents
- ▶Cancer, Molecular Mechanisms of Therapy

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Antimicrobial Agents

Antimicrobial drugs are used for the treatment of diseases caused by microorganisms (bacterial or viral infections).

- ▶β-Lactam Antibiotics
- ▶Ribosomal Protein Synthesis Inhibitors
- ▶Quinolons
- ▶Antiviral Drugs
- ▶Microbial Resistance to Drugs

Antimode

The antimode is the cut-off value separating different functionally defined groups in a bi-modal or multi-modal frequency distribution.

- ▶Pharmacogenetics

Antimycotic Drugs

- ▶Antifungal Drugs

Antineoplastic Agents

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Synonyms

Anticancer drugs; Cytostatic drugs; Cytotoxic drugs; Antitumor drugs

Definition

Cancer or neoplastic disease is a genomic disorder of the body's own cells which start to proliferate and metastasize in an uncontrolled fashion that is ultimately detrimental to the individual. Antineoplastic agents are used in conjunction with surgery and radiotherapy to restrain that growth with curative or palliative intention. The domain of antineoplastic chemotherapy is cancer that is disseminated and therefore not amenable to local treatment modalities such as surgery and radiotherapy.

Development and Characteristics of Cancer Cells

The genesis of cancer cells can be modeled by the formula

$$\text{Cancer} = f\{\text{Exposure, Genetic disposition, Age}\}$$

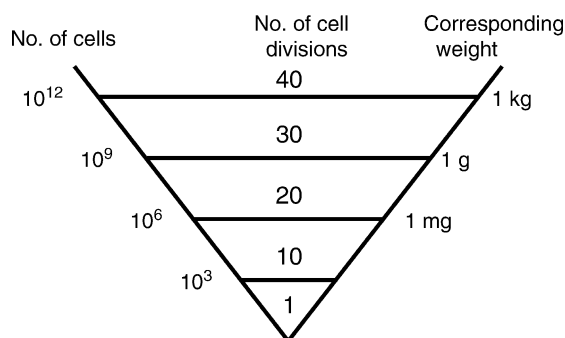
in which "*Exposure*" denotes the impact of exogenous factors that can be of chemical (chemical carcinogens), physical (UV or γ -irradiation) or biological (viruses, bacteria) origin. "*Genetic disposition*" indicates the germline transmission of genes associated with cancer development and "*Age*" points to the fact that certain cellular injuries which cause mutations and lead to cancer development are not reversible but accumulate with time. Thus, a cancer cell is characterized by genetic abnormalities such as chromosomal alterations, changes in its DNA- methylation status as well as activation of cellular protooncogenes (mainly related to cellular growth) to oncogenes and inactivation of tumor suppressor genes. As result of these changes they show autonomous proliferation, dedifferentiation, loss of function, invasiveness, and metastasis formation. Furthermore, drug resistance (primary or acquired in response to treatment with antineoplastic drugs) is a common phenomenon.

Growth of Cancer Cells

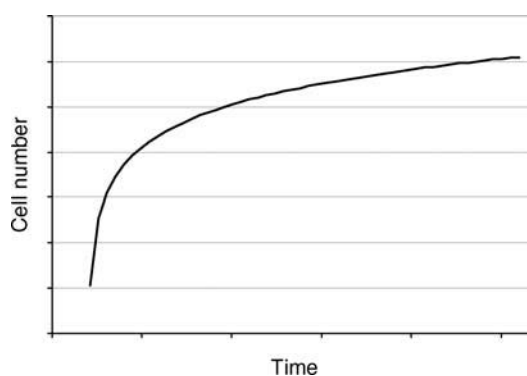
Proliferation of cancer cells is not restricted by **►contact inhibition** as for normal cells but rather by the supply of growth factors and nutrients. Once a cell has become malignant and its descendants got the necessary blood supply, the initial growth rate is exponential and follows approximately the pattern shown in Fig. 1. The subsequent loss of logarithmic growth is related to insufficient supply of nutrients which drives cancer cells to either die or exit the cell cycle. As result, larger tumors contain only a certain ratio of dividing cells which is termed growth fraction. The resultant steady state growth is depicted by the so called Gompertz function (Fig. 2).

Mechanism of Action

Anticancer drugs can be grouped into several classes, according to their mechanism of action and origin [1]. These are (i) alkylating agents and related compounds which act by forming covalent bonds with cellular



Antineoplastic Agents. Figure 1 Relationship of cell number, number of cell divisions, and corresponding weight.



Antineoplastic Agents. Figure 2 Growth curve of tumor cells according to Gompertz.

macromolecules such as DNA, (ii) antimetabolites which block metabolic pathways that are vital for cell survival or proliferation, (iii) cytotoxic antibiotics of microbial origin and (iv) plant derivatives which mainly interfere with mammalian cell division, (v) hormonal agents which suppress hormone secretion, block hormone synthesis or antagonize hormone action, (vi) biological response modifiers which enhance the host's response to cancer cells, (vii) antibodies which recognize antigens specific for cancer cells, (viii) tyrosine kinase inhibitors, (xi) antisense oligonucleotides, and (x) miscellaneous agents which do not fit into the classes described above [2].

General effects include cytostatic or cytotoxic effects, the latter being related to killing a constant fraction of cells. The mode of action of antineoplastic agents is not causal for it does not reverse the basal changes that have led to the development of cancer cells but symptomatic since it aims at their destruction, or more recently, on blocking/ablating the result of genetic alterations by inhibiting proteins whose malfunction contributes to the formation and maintenance of cancer cells. However, cytotoxicity is generally not restricted towards cancer cells but for the

majority of currently used, classical antineoplastic drugs (groups 1–4) affects all (quickly) dividing cells, especially those from bone marrow, gastrointestinal tract, hair follicles, gonads, and growing tissues in children (lack of selectivity).

The limited efficacy of classical anticancer drugs can be explained in part by the compartment model of dividing (growth fraction, compartment A) and nondividing (compartment B) cells. The majority of antineoplastic drugs acts upon cycling cells and will hit, therefore, compartment A only.

Alkylating Agents and Related Compounds

Alkylating agents are activated spontaneously or enzymatically to give rise to an electrophilic species that can form covalent bonds with nucleophilic cellular constituents. Reaction of monofunctional agents with DNA bases will lead to single strand breaks, that of bifunctional groups to **crosslinks** between bases of the same strand of DNA (intrastrand crosslink) or two complementary strands (interstrand crosslink). Replicating cells are more susceptible to these drugs since parts of the DNA are unpaired and not protected by proteins. Therefore, although alkylating agents are not cell cycle specific, cells are most susceptible to alkylation in late G1- and S-phases of the cell cycle resulting in block at G2 and subsequent apoptotic cell death.

Cells which survive these damages may have undergone mutations leading themselves to cancer development. This is reason for the carcinogenicity of alkylating agents.

The most important subgroup is that of nitrogen mustard derivatives. Nitrogen mustard was developed in relation to sulfur mustard, the “mustard gas” used during World War I which was found to suppress **leukopoiesis**. Nitrogen mustard was the first drug to induce a remission in a lymphoma patient at the end of World War II, but has been abandoned since then. The highly reactive R-N-bis-(2-chloroethyl) group, however, is part of many drugs in current use, such as cyclophosphamide, melphalan, and chlorambucil. The activity of cyclophosphamide is dependent on P450 mixed function oxidases mediated activation into active and/or toxic metabolites. One of the toxic metabolites is acrolein which causes hemorrhagic cystitis if not prevented by the antidote mesna. Mesna is a sulfhydryl donor and interacts specifically with acrolein, forming a nontoxic compound.

Other subgroups of alkylating agents are the nitrosoureas (examples: carmustine, BCNU; lomustine, CCNU) and the triazenes (example: dacarbazine, DTIC). Platinum derivatives (cisplatin, carboplatin, oxaliplatin) have an action that is analogous to that of alkylating agents (formation of crosslinks) and therefore are appended to this class, as well.

Alkylating agents are used for treating solid tumors as well as leukemias and lymphomas. Their broad spectrum of activity is reason for their inclusion into many past and current chemotherapy schedules. Platinum derivatives are especially useful in treating testicular and ovarian cancers. All alkylating agents depress bone marrow function and cause gastrointestinal side effects. With prolonged use depression of gametogenesis will result leading to sterility and an increased risk of leukemias as well as other malignancies.

Antimetabolites

Antimetabolites interfere with normal metabolic pathways. They can be grouped into folate antagonists and analogues of purine or pyrimidine bases. Their action is limited to the S-phase of the cell cycle and therefore they target a smaller fraction of cells as compared with alkylating agents.

The main folate antagonist is methotrexate. In structure, folates are based on three elements: a heterobicyclic pteridine, *p*-aminobenzoic acid and glutamic acid. The latter moiety is polyglutamated within cells which causes a prolonged intracellular half life and, as compared with monoglutamate, an increased affinity to dihydrofolate reductase, the target enzyme of antifolates. This enzyme catalyses the reduction of dihydrofolate to tetrahydrofolate and its inhibition interferes with the transfer of monocarbon units that are needed for purine- and thymidylate synthesis and thus blocks the synthesis of DNA, RNA, and Protein. Methotrexate has a higher affinity for dihydrofolate reductase than the normal substrate (dihydrofolate) and inhibits thymidylate synthesis at a tenfold lower concentration (1 nM) than purine synthesis. Methotrexate is toxic to normal tissues (especially the bone marrow) and causes hepatotoxicity following chronic therapy. Acute toxicity following iatrogenic error or high dose therapy can be rescued by using folinic acid, a form of tetrahydrofolate, as antidote.

Purine- and pyrimidine analogs are characterized by modifications of the normal base or sugar moieties. The uridine analog 5-fluorouracil is converted intracellularly into fluorouridine-monophosphate (FUMP) and fluorodeoxyuridine-monophosphate (FdUMP). Further phosphorylation leads to the respective triphosphates FUTP and FdUTP. FdUMP inhibits the enzyme thymidylate synthase and thus blocks the generation of thymidine whereas FUTP and FdUTP are incorporated into RNA and DNA, respectively. Gemcitabine is a cytosine analog in which the pentose moiety contains two fluorine atoms at position 2 of the sugar ring. This drug is converted into the respective diphosphate, which inhibits ribonucleotide reductase, and the triphosphate, which after incorporation into DNA causes masked termination of DNA chain elongation since the altered base sequence cannot be

efficiently repaired. Cytosine arabinoside is a cytosine analog with a “wrong” pentose which after phosphorylation to the respective triphosphate inhibits the DNA polymerase. The purine analogs mercaptopurine and fludarabine are converted into fraudulent nucleotides and inhibit DNA polymerase. The main unwanted effects are gastrointestinal epithelial cell damage and myelotoxicity.

Cytotoxic Antibiotics

Cytotoxic antibiotics affect normal nucleic acid function by intercalating between DNA bases which blocks reading of the DNA template and also stimulate ►topoisomerase II dependent DNA-double strand breaks. In addition, metabolism of the drugs gives rise to free radicals which cause DNA damage and cytotoxicity, and to membrane effects that occur directly or via oxidative damage. Cytotoxic antibiotics are poorly absorbed from the gut and therefore are given intravenously. They have long half-lives (1–2 days), and are eliminated by metabolism.

Anthracyclins (e.g., doxorubicin, epirubicin, and idarubicin) are the most important subgroup. They consist of a four ringed planar quinone structure attached to an amino sugar group. In addition to the general unwanted effects, anthracyclins can cause cumulative, dose-related cardiotoxicity leading to heart failure and hair loss. Epirubicin is less cardiotoxic than doxorubicin.

Mitoxantrone has a three ringed planar quinone structure with amino containing side chains and exerts also dose-related cardiotoxicity and bone marrow depression. Mitomycin C is a nonplanar tricyclic quinone which is activated to give an alkylating metabolite. Bleomycins are metal chelating glycopeptides that degrade DNA, causing chain fragmentation and release of free bases. This subgroup causes little myelosuppression but pulmonary fibrosis, mucocutaneous reactions, and hyperpyrexia. Actinomycin D (dactinomycin) is a chromopeptide which intercalates in the minor groove of DNA between adjacent guanosine–cytosine pairs and interferes with RNA polymerase, thus preventing transcription. Unwanted effects include nausea, vomiting, and myelosuppression.

In general, the mechanisms of action are not cell cycle specific, although some members of the class show greatest activity at certain phases of the cell cycle, such as S-phase (anthracyclins, mitoxantrone), G1- and early S-phases (mitomycin C) and G2- and M-phases (bleomycins).

Plant Derivatives

Plant derivatives comprise several subgroups with diverse mechanisms of action.

Some are mitosis inhibitors which affect microtubule function and hence the formation of the mitotic spindle, others are topoisomerase I and II inhibitors.

Vinca alkaloids (vincristine, vinblastine, vindesine) are derived from the periwinkle plant (*Vinca rosea*), they bind to tubulin and inhibit its polymerization into microtubules and spindle formation, thus producing metaphase arrest. They are cell cycle specific and interfere also with other cellular activities that involve microtubules, such as leukocyte phagocytosis, chemotaxis, and axonal transport in neurons. Vincristine is mainly neurotoxic and mildly hematotoxic, vinblastine is myelosuppressive with very low neurotoxicity whereas vindesine has both, moderate myelotoxicity and neurotoxicity.

Taxanes (paclitaxel, docetaxel) are derivatives of yew tree bark (*Taxus brevifolia*). They stabilize microtubules in the polymerized state leading to nonfunctional microtubular bundles in the cell. Inhibition occurs during G2- and M-phases. Taxanes are also radiosensitizers. Unwanted effects include bone marrow suppression and cumulative neurotoxicity.

Epidodophyllotoxins (etoposide, teniposide) are derived from mandrake root (*Podophyllum peltatum*). They inhibit topoisomerase II thus causing double strand breaks. Cells in S- and G2-phases are most sensitive. Unwanted effects include nausea and vomiting, myelosuppression, and hair loss.

Camptothecins (irinotecan, topotecan) are derived from the bark of the Chinese tree Xi Shu (*Camptotheca accuminata*). They inhibit topoisomerase I thus effecting double strand breaks. Unwanted effects include diarrhea and reversible bone marrow depression.

Hormonal Agents

Tumors derived from hormone sensitive tissues may remain hormone dependent and are then amenable to therapeutic approaches with hormonal agents. These include hormones with opposing (apoptotic) action, hormone antagonists, and agents that inhibit hormone synthesis.

Glucocorticoids have inhibitory (apoptotic) effects on lymphocyte proliferation and are used to treat leukemias and lymphomas. Estrogens (fosfestrol) are used to block the effect of androgens in prostate cancer. Progestogens (megestrol, medroxyprogesteroneacetate) have been useful for treating endometrial carcinoma, renal tumors, and breast cancer.

Gonadotropin releasing hormone analogs (goserelin, buserelin, leuprorelin, triptorelin) inhibit gonadotropin release and thus lower testosterone or estrogen levels. They are used to treat breast cancer and prostate cancer.

Hormone antagonists (tamoxifen and toremifen bind to the estradiol receptor, flutamide binds to the androgen receptor) are used for treating breast and prostate cancer.

Aromatase inhibitors (aminoglutethimide, formestane, trilostane) block the formation of estrogens from precursor steroids and thus lower estrogen levels. They have been used for treating breast cancer.

Side effects are less prominent in type and extent as compared with cytostatics and include typical hormonal or lack of hormone like effects.

Biological Response Modifiers

Agents which enhance the host's response against neoplasias or force them to differentiate are termed biological response modifiers. Examples include interleukin 2 which is used to treat renal cell carcinoma, interferon α which is active against hematologic neoplasias, and tretinoin (all-trans retinoic acid) which is a powerful inducer of differentiation in certain leukemia cells by acting on retinoid receptors. Side effects include influenza like symptoms, changes in blood pressure and edema.

Antibodies

Recombinant ► **humanized monoclonal antibodies** have been used recently to target antigens that are preferentially located on cancer cells. Examples include trastuzumab and rituximab which are used to treat HER2 positive breast cancer and B-cell type lymphomas, respectively. Unwanted side effects include anaphylactic reactions.

A more recently developed IgG1 monoclonal antibody is cetuximab which targets the epidermal growth factor receptor (EGFR). This binding inhibits the activation of the receptor and the subsequent signal-transduction pathway, which results in reducing both the invasion of normal tissues by tumor cells and the spread of tumors to new sites. Cetuximab is used to treat colorectal cancer and locally advanced squamous cell carcinoma of the head and neck. The most common side effects include an acne-like skin rash that seems to be correlated with a good response to therapy and hypersensitivity reactions.

Another monoclonal antibody is bevacizumab which binds to and inhibits the activity of vascular endothelial growth factor (VEGF) thus inhibiting the interaction of VEGF with its receptor on endothelial cells. This, in turn, inhibits the proliferation of endothelial cells and the formation of new blood vessels. In essence then, it kills tumors by cutting off its own blood supply. For this activity bevacizumab belongs to a family of drugs termed antiangiogenic agents, or angiogenesis inhibitors. Bevacizumab is used to treat metastatic colorectal cancer and nonsmall cell lung cancer (NSCLC). Side effects include hemorrhage, hypertension, gastrointestinal perforation/wound healing complications, and congestive heart failure.

Tyrosine Kinase Inhibitors/Receptor Associated Tyrosine Kinase Inhibitors (RTK-I)

A number of anticancer drugs have been developed that specifically target kinases known to be oncogenic. The first drug in this area is imatinib mesylate, which targets

ABL, *KIT*, and *PDGFR*. Imatinib mesylate is the treatment of choice for patients with chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST). In CML, the Philadelphia chromosome (Ph) results from a translocation which codes for the chimaeric fusion protein, BCR-ABL, which is a constitutively activated tyrosine kinase. Imatinib inhibits the normal Abelson tyrosine kinase (ABL) as well as BCR-ABL. The protooncogene *c-KIT* encodes the KIT tyrosine kinase, which serves as a receptor for stem cell factor. KIT is important in cell cycle regulation and critically important in haematopoiesis. GIST tumors contain a mutated *c-kit* gene leading to increased activity of this tyrosine kinase. Imatinib mesylate blocks the activity of c-KIT and thus suppresses tumor cell proliferation. Also, two receptors for platelet-derived growth factor (PDGF) are sensitive to imatinib. PDGF is involved in cell cycle regulation, angiogenesis, and fibroblast proliferation. Side effects of imatinib mesylate are rare.

Other drugs of this class include gefitinib (iressa®) and erlotinib (tarceva®) which attach to the EGF receptor, prevent the receptor from being activated and thus stop the cells from dividing. Both agents have been used in patients with nonsmall cell ► **lung cancer**. Side effects include diarrhoea, acne-like rash, loss of appetite, nausea and vomiting, tiredness, and change in blood pressure. Sorafenib is a multitargeted kinase inhibitor, which blocks the activity of VEGF. Sorafenib is used to treat ► **kidney cancer**, side effects include hand/foot skin reaction, effects on the skin, and high blood pressure.

Antisense Oligonucleotides

Oblimersen sodium is a DNA antisense oligonucleotide designed to specifically bind to human bcl-2 mRNA, resulting in catalytic degradation of bcl-2. This results in decreased translation of the protein Bcl-2, which is a cellular antiapoptotic protein. Thus, oblimersen enhances sensitivity to chemotherapy by shifting the intracellular balance to a state in which the cells are more likely to be killed by apoptosis. Currently, it is used in combination chemotherapy for treating advanced melanoma.

Miscellaneous Agents

Antineoplastic agents that cannot be grouped under subheadings 1–9 include miltefosine which is an alkylphosphocholine that is used to treat skin metastasis of breast cancer, and crispatase which breaks down asparagine to aspartic acid and ammonia. It is active against tumor cells that lack the enzyme asparaginase, such as acute lymphoblastic leukemia cells. Side effects include irritation of the skin in the case of miltefosine and anaphylactic reactions in the case of crispatase. Another recent development is the proteasome inhibitor bortezomib which is used to treat multiple ► **myeloma**.

The ►boron atom in bortezomib binds the catalytic site of the ►26S proteasome with high affinity and specificity. In normal cells, the proteasome regulates protein expression and function by degradation of ubiquitinated proteins, and also cleanses the cell of abnormal or misfolded proteins. While multiple mechanisms are likely to be involved, cancer cells may be especially susceptible to proteasome inhibition since more abnormal or misfolded proteins are likely to be present. Also, bortezomib may prevent degradation of proapoptotic factors, permitting activation of programmed cell death in neoplastic cells dependent upon suppression of proapoptotic pathways. Main side effects include nausea and vomiting, fatigue, and diarrhea.

Clinical Use

Cancer treatment is a multimodality treatment, i.e., surgery is combined with radiotherapy and antineoplastic chemotherapy. The latter treatment mode is used mainly for cancers which have disseminated. Different forms of cancer differ in their sensitivity to chemotherapy with antineoplastic agents. The most responsive include lymphomas, leukemias, choriocarcinoma and testicular carcinoma, while solid tumors such as colorectal, pancreatic and squamous cell bronchial carcinomas generally show a poor response. The clinical use of antineoplastic agents is characterized by the following principles.

1. The therapeutic ratio of antineoplastic agents, which is defined by the dose necessary to cause a significant anticancer effect divided by the dose effecting significant side effects, is generally low (near to one).
2. The intention to treat a cancer patient can vary between curative and palliative, pending on the prognosis. Antineoplastic therapy with curative intention is based on high dosages and takes into account severe side effects that have to be tolerated by patients in order to receive the optimal treatment. Palliative therapy with cytotoxic agents aims at maximum life quality for a patient who can not be cured. This includes palliation of symptoms like pain, fractures, and compression of vital tissues that are caused by cancer growth, but tries to accomplish this aim with dosages of cytostatics that bring about as few side effects as possible.
3. Generally, combination therapy with antineoplastic agents is superior to monotherapy. The reason is that several different mechanisms of action can be combined thus lowering the risk of rapid induction of resistance, and the dosages of the single agents can be reduced. This, in turn, decreases the incidence in side effects caused by the single agents and, in addition, the side effects will not sum up if the respective toxicity profiles differ from each other.

4. To be successful, antineoplastic therapy often has to be applied for considerable periods of time. The initial therapy period is being termed “induction therapy” which is then followed by a “maintenance therapy” and possibly a “reinduction therapy.”
5. The therapeutic success is measured by its effect on tumor size and can be described as tumor remission (complete or partial), stable disease, or progression of the tumor. Also, the impact of a therapy is related to time and can be measured as disease free interval, time to progress, or overall survival time.
6. Patients receiving cytotoxic chemotherapy very often need concomitant administering of antiemetic therapy. Such protocols will start well in advance of administering the cytotoxic, and last for a reasonable time with regard to pharmacokinetics of the antineoplastic agent. In addition, side effects of antineoplastic therapy are made better tolerable by supportive care.
7. Few side effects can be alleviated by the use of antidotes. An example is the prevention of hemorrhagic cystitis caused by cyclophosphamide by the concomitant infusion of mesna.

►Cancer, Molecular Mechanisms of Therapy

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Anti-obesity Drugs

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Synonyms

Weight-loss therapies

Definition

►Obesity results from an energy imbalance, when energy intake exceeds energy expenditure over a prolonged period of time. The excess energy is stored in the form of triglycerides in the adipose tissue.

Obesity is defined by the body mass index (BMI), measured as body weight in kilograms divided by the squared height in metres (kg/m^2). A BMI of 30 or more is a commonly-used criterion for defining obesity and a BMI between 25 and 30 is considered overweight.

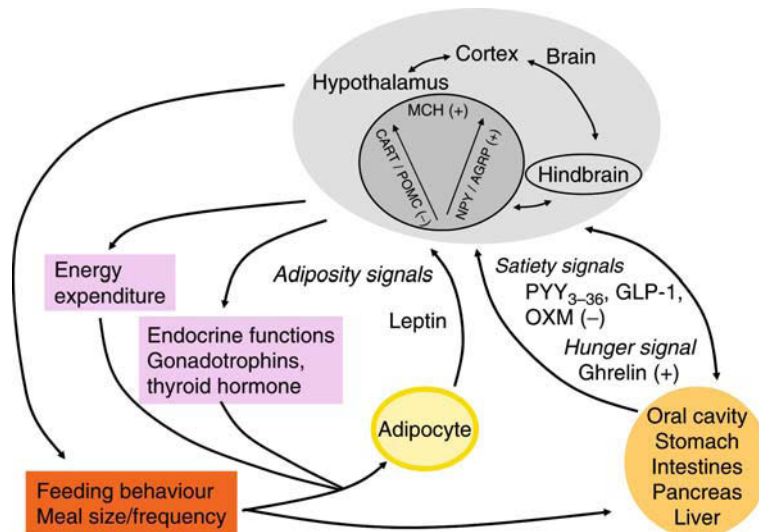
Energy homeostasis depends on energy intake and energy expenditure. The main components determining energy intake are appetite and intestinal absorption, while energy expenditure depends on thermogenesis and metabolism. In theory, anti-obesity therapies could aim to modify energy intake, energy expenditure and/or energy storage. The current anti-obesity drugs on the market and most of those used in clinical development reduce energy intake, either acting centrally (appetite suppressant) or peripherally (reduction of fat absorption). The progress in the field of modulating energy expenditure is not so advanced.

Reduction of energy intake: appetite and satiation
Reduction of energy intake: intestinal absorption
Increase of energy expenditure
Modulation of fat storage

Mechanism of Action

Regulation of Energy Balance and Food Intake

Energy homeostasis, food intake and energy stores are regulated in a complex manner by central and peripheral pathways that are built into short-term and long-term feedback loops (Fig. 1). The hormone leptin plays a central role in the regulation of energy balance. It is an adipocyte-derived factor, or ►**adipokine**, which is released from adipose tissue into the circulation and informs the brain about the status of energy stores. The blood levels of leptin are positively correlated with adipocyte number and size. Binding of leptin to specific leptin receptors in the ►**hypothalamus** initiates a cascade that ultimately regulates feeding behaviour, neuroendocrine functions (gonadotrophins, thyroid axes) and energy expenditure (via the sympathetic nervous system). Leptin has thus broad effects on energy balance, which ultimately modulate the size of the energy/fat stores. Adiponectin, another ►**adipokine**, and the pancreatic hormone ►**insulin** have been suggested as additional mediators of the energy status between periphery and the CNS.



Anti-obesity Drugs. Figure 1 Regulation of energy/fat stores and feeding behaviour by central and peripheral mechanisms. A central mediator is leptin, which informs the brain about the status of energy reserves in the adipose tissue. Leptin is released in correlation with adipocyte number and size and binds to its receptors in the hypothalamus to initiate a cascade that ultimately regulates feeding behaviour, neuroendocrine functions (gonadotrophins, thyroid hormone, etc) and energy expenditure. As a result, energy/fat stores are affected and controlled in the long-term. Adiponectin may have a similar role as a mediator between the periphery and CNS. Elevated leptin levels reflecting increased energy stores, downregulate the expression of appetite-stimulating (orexigenic) peptides neuropeptide Y (NPY) and agouti-related protein (AGRP) and stimulate the expression of the anorexigenic peptides pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). The activity of these neuropeptides influences the sensitivity to signals from the gastrointestinal (GI) tract. Signals from the GI tract are involved mainly in the short-term regulation, i.e. the meal-to-meal regulation of appetite by inducing hunger signals (e.g. ghrelin) or satiety (e.g. PYY₃₋₃₆, OXM (oxyntomodulin), GLP-1 (Glucagon-like peptide-1)) and include sensors of gastric distention. The short-term and long-term signals are finally integrated by the brain. (+) orexigenic, (-) anorexigenic effects.

Leptin exerts its effects on food intake behaviour via two distinctive neuronal populations in the hypothalamus. Elevated levels of leptin inhibit neurons expressing the appetite-stimulating (►**orexigenic**) peptides ►**neuropeptide Y** (NPY) and agouti-related protein (AGRP) and stimulate neurons expressing the appetite-suppressing (►**anorexigenic**) peptides pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), which are co-localized in a different neuron population. POMC is the precursor of α -melanocyte-stimulating hormone that activates the melanocortin-4 receptor to mediate anorexigenic effects. The neuron populations project further to other brain centres, which ultimately communicate with the cerebral cortex for the final coordination of feeding. Neuropeptides further downstream from leptin include ►**galanin**, melanin concentrating hormone (MCH) and the ►**orexins** (also termed hypocretins), which all exert orexigenic effects. The level of food intake will in turn affect fat depots, thus completing the feedback loop between the periphery and CNS.

The ►**endocannabinoid** system (ECB) system was recently shown to regulate appetite and energy homeostasis. Endocannabinoids are endogenous lipids derived from arachidonic acid that are capable of binding to and activating the two ►**G-protein coupled** receptors cannabinoid receptor CB1 and CB2. The regulation of food intake and peripheral effects are thought to be mediated via CB1 expressed in the CNS and in various peripheral tissues, including adipose tissue, the gastrointestinal tract, reproductive tissue, muscle and the liver. The 'first-in class' CB1 receptor antagonist rimonabant (Table 1) has recently been approved for anti-obesity treatment.

Sibutramine (Table 1) has been in the market for several years and inhibits the reuptake of serotonin, noradrenaline and, to a lesser extent, dopamine. It acts

mainly as an appetite suppressant and may also increase energy expenditure.

Feeding behaviour, defined by frequency and size of meals, is also regulated in a short-term loop. During and after a meal, various signals are generated in the periphery including taste signals from the oral cavity, gastric distension and humoral signals from secretory cells of the gastrointestinal (GI) tract. These afferent signals are transmitted to the hypothalamus and the hindbrain, which communicate with higher brain areas. Several GI hormones were shown not only to regulate appetite and satiety but also to improve the response of the pancreas to absorbed nutrients (►**incretin hormones**). GI hormones have gained renewed interest, with the most recent example being peptide YY (PYY). PYY₃₋₃₆ is secreted from the distal gut in response to food ingestion. It belongs to the family of peptides including NPY and pancreatic polypeptide (PP), which exert their effects via the ►**G-protein-coupled Y** receptor family, Y₁–Y₅. Initial studies showed that peripheral administration of PYY₃₋₃₆ reduced food intake, supposedly mediated via Y₂ receptors, however the potential as an anti-obesity drug is as yet unclear. The related peptide PP is released postprandially from the pancreas in a biphasic manner and is also being investigated for its anorexigenic effects. Glucagon-like peptide-1 (GLP-1), which results from processing of the pre-pro-glucagon precursor in the pancreas, intestinal cells and CNS, presumably regulates feeding by both peripheral and central mechanisms by delaying gastric emptying. The satiety peptide oxyntomodulin (OXM) is derived from the same precursor. Cholecystokinin (CCK) has also been proposed as satiety factor. The only gut hormone identified so far with orexigenic effects is ghrelin. This peptide was originally described as a growth hormone segretagogue. Ghrelin is mainly produced in the stomach, released into the blood, and reaches growth hormone segretagogue receptors in the hypothalamus.

Anti-obesity Drugs. Table 1 Profile of drugs approved for long-term treatment of obesity

	Orlistat	Sibutramine	Rimonabant
Site of action	Gut	CNS	CNS/periphery
Molecular target	Gastrointestinal lipases	Serotonin and noradrenaline transporter	Cannabinoid-1 receptor
Mode of action	Enzyme inhibition	Reuptake inhibition	Receptor antagonism
Effect	Reduced fat absorption	Appetite suppression	Appetite suppression, peripheral actions
Additional effects	LDL-C reduction	Increased energy expenditure?	Metabolic effects
Unwanted/side effects	GI effects	CV system	CNS?
Daily dosage	3 × 120 mg with meals	1 × 10 or 15 mg	1 × 20 mg

It was shown to decrease the production of leptin and NPY and reduced food intake when administered to humans and rodents.

Several GI peptides or derivatives are under investigation for their potential use as anti-obesity therapy.

Reduction of Energy Intake: Inhibition of Absorption

Inhibition of the absorption of fat (triglycerides) in the GI lumen represents the most efficient approach for reduction of caloric intake, as triglycerides are the most condensed energy stores. In the intestine, triglycerides are split into free fatty acids (FFAs) and monoglycerides by lipases, the targets for orlistat (Table 1). After hydrolysis, FFAs cross the membranes of the epithelial cells lining the intestinal wall. Once inside the epithelial cell, FFAs are donated to acyl-CoA synthetase in the endoplasmic reticulum by fatty acid-binding proteins (FABPs). Acyl-CoA is then transferred to 2-monoacylglycerol to resynthesize triglycerides. Acyl-CoA:diacylglycerol acyltransferase (DGAT) is a key enzyme responsible for the final step in the glycerol phosphate pathway of triglyceride synthesis. The absorption of dietary fat thus involves several steps catalysed by proteins that might represent promising drug targets.

Increase of Energy Expenditure

Total energy expenditure is the sum of basal metabolism, i.e. the constant obligatory energy expenditure required for cell and organ survival, and a variable portion needed for physical activity and adaptive thermogenesis. The stimulation of thermogenesis has raised much interest as a possible mechanism to treat obesity, especially once the mitochondrial uncoupling proteins (UCPs) were identified. UCP1 is selectively expressed in brown adipose tissue (BAT), which is rich in mitochondria and highly developed in rodents for thermogenesis. Activation of the sympathetic nervous system in response to cold stress or high-fat diet activates β 3-adrenoreceptors of the BAT, resulting in increased cAMP levels and stimulation of protein kinase A. This kinase phosphorylates and activates hormone-sensitive lipase, thereby promoting the release of FFAs. These serve both as fuel for mitochondrial respiration and as activators of UCP1. UCP1 dissipates the transmembrane proton gradient coupled to the oxidation of metabolites, releasing energy as heat. In addition, there is a chronic response, i.e. UCP1 is transcriptionally upregulated, and mitochondrial biogenesis is stimulated through mechanisms involving a transcriptional coactivator of the nuclear peroxisome proliferator-activated receptor- γ ($\gg$ PPAR γ), with the acronym PGC-1 (PPAR γ coactivator-1).

Despite tremendous efforts, research on energy expenditure did not yet lead to successful therapies. Much attention has been devoted to the stimulation of β 3 receptors. The stimulation of these adrenergic receptors should lead to an increased expression of the

uncoupling protein UCP1, thereby increasing thermogenesis, and inducing catecholamine-induced lipolysis. The first generation of β 3 receptors agonists failed due to species differences between human and rodent receptors. A general problem of this therapeutic approach in human may be the lack of BAT, which is prominent in rodents but disappears in human after birth, while the major thermogenic tissue in man is skeletal muscle. As UCP3 is expressed in skeletal muscle in man, it has been proposed as a promising pharmacological target, but its definite role is still uncertain.

Modulation of Fat Storage

Processes involved in the storage of fat, including adipocyte differentiation, angiogenesis or apoptosis, could also be targeted as a way to reduce fat mass. However, all of these potential approaches to reduce the ability to synthesize or store fat will be safe only if associated with an increase in fat oxidation and/or with a reduction of fat absorption. Otherwise, the inability to deliver excess calories to adipose tissue could have serious secondary consequences as lipids accumulate in the blood or various organs. A safer anti-obesity approach could be the stimulation of BAT formation in man, involving either de novo recruitment from pre-adipocytes or interconversion of white adipose tissue, which is the major site for triglyceride storage.

Clinical Use (Including Side Effects)

Obesity has reached an epidemic level not only in developed but also in developing countries. In the US, ~65% of the adult population is overweight and 20–25% of these are obese. Obesity is a complex condition frequently associated with other diseases such as type 2 $\gg$ diabetes and hypertension, which makes it a major health issue. The treatment of obesity aims at a sustained loss of 5–10% of body weight, which has been shown to reduce the risk of obesity-associated co-morbidities. Anti-obesity therapy should result in a reduction of the fat mass while saving lean body mass, and help to maintain the reduced weight. Furthermore, an anti-obesity drug should not induce counterregulatory mechanisms which limit its efficacy during long-term treatment, and it needs to meet high safety standards.

There are three medications approved for the long-term (>6 months) treatment of obesity: (i) Orlistat, an inhibitor of fat absorption, (ii) Sibutramine, an appetite suppressant and (iii) the recently approved Rimonabant, an appetite-suppressing agent with additional peripheral action. These medications are recommended to be used in conjunction with a reduced caloric diet (or reduced fat diet for orlistat), and increased exercise. Several other drugs are approved for short-term treatment of obesity only and are either catecholaminergic or serotonergic CNS-active (activating the sympathetic nervous system) anorectic agents (e.g. phentermine).

Orlistat (Xenical[®], Reductil[®]) – Lipase Inhibitor

Orlistat inhibits gastrointestinal lipases in the lumen of the GI tract to decrease systemic absorption of dietary fat. It is a hydrogenated derivative of lipstatin, a natural occurring lipase inhibitor of bacterial origin. The drug binds covalently to a serine residue in the active site of gastrointestinal lipases, and thus inhibits the hydrolysis of ingested triglycerides into absorbable FFAs and monoglycerides. At doses of 400–600 mg daily, ~30% of triglycerides are not absorbed by the small intestine and excreted into faeces, thereby contributing to the caloric deficit. Orlistat represents an overall safe treatment for obesity, given that the drug itself is minimally absorbed.

A clinical trial with orlistat in conjunction with a hypocaloric diet showed a weight loss of 8.7 kg in patients receiving orlistat versus 5.8 kg in patients receiving placebo after 52 weeks of treatment. The effect on body weight was sufficient to improve several metabolic parameters, including reduced LDL-C blood levels, improved oral glucose tolerance and blood pressure. The main unwanted effects of orlistat are attributable to its mode of action, as non-digested fat remains in the intestinal lumen and can cause steatorrhoea (fatty stools), flatulence and faecal incontinence. These effects are associated with a high-fat meal, and therefore a low-fat diet is recommended. As absorption of fat-soluble vitamins may be hampered, supplementation of these vitamins is recommended.

Sibutramine (Meridia[®]) – Serotonin and Noradrenaline Reuptake Inhibitor

Sibutramine is a β -phenylethylamine derivative that inhibits the reuptake of noradrenaline, serotonin, and, to a lesser extent, dopamine in the CNS, resulting in reduced hunger and increased satiety. It may also increase thermogenesis, causing an increase in energy expenditure. Sibutramine treatment is indicated for weight loss and maintenance medication. A meta-analysis of randomized placebo-controlled trials with doses of 10–20 mg per day over 44–54 weeks indicated an average weight loss of 4.45 kg. Several metabolic parameters were also improved. However, there are some safety concerns as increase in systolic and diastolic blood pressure and a rise in heart rate have been observed. These cardiovascular effects of sibutramine may be explained as a consequence of the drug's peripheral effects, i.e. the inhibition of noradrenaline reuptake at sympathetic nerve terminals in the arterioles. Sibutramine should therefore be used with caution in patients with poorly controlled hypertension or with a history of cardiovascular heart disease.

Rimonabant (Acomplia[®]) – CB1 Receptor Antagonist

Rimonabant is the 'first-in class' CB1 receptor antagonist, which has recently reached the market in Europe (approval in the US is pending). The reduction of

weight observed in the clinical trials is of the same order of magnitude as that seen with the other available agents sibutramine and orlistat. In the 'Rimonabant in Obesity'-lipid trial, HDL cholesterol was increased, plasma triglycerides reduced, and glucose tolerance upon oral glucose challenge improved. Rimonabants' beneficial effects on risk factors are supposedly not only attributed to the weight loss induced by its appetite suppressant effect but also mediated through peripheral effects. Observed side effects in the trials consisted mainly of nausea, dizziness and anxiety.

Comment on the First Leptin Trials

Leptin has proved to be an efficient treatment for the rare form of obesity associated with leptin deficiency. By contrast, the results of the first clinical trial with human leptin in obese patients (without leptin deficiency) were less promising. This may be explained by leptin resistance in a high proportion of these patients. However, the mechanisms involved in the development of leptin resistance could become new drug targets.

- Appetite Control
- Adipokines
- Diabetes Mellitus

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Antioncogene

An antioncogene is a gene that suppresses cellular proliferation.

- Targeted Cancer Therapy

Antioxidants

Antioxidants are substances which reduce or prevent the oxidation of other molecules. While oxidation reactions

are important for the basic metabolism of cells, they can also be damaging under certain conditions. One of the major sources for reactive oxygen is the leakage of activated oxygen like superoxide (O_2^-) which is produced via the respiratory chain in mitochondria. Also a variety of enzymes like xanthine oxidase, P450 oxidases, FADH₂ oxidase, or NADPH oxidase can produce superoxide. However, under normal conditions most cells maintain a reducing environment due to the activity of various anti-oxidant mechanisms. Several enzymes such as superoxide dismutase, glutathione peroxidase, or catalase can convert superoxide to hydrogen peroxide (H_2O_2) and finally to water. There are also various anti-oxidants such as ascorbic acid (vitamin C), glutathione, or α -tocopherol (vitamin E) which are present in cells and inactivate reactive oxygen species like superoxide or hydrogen peroxide. Under various pathological conditions, however, the balance between the formation of reactive oxygen species and their inactivation by anti-oxidant enzymes and substances can be dysregulated resulting in the increased accumulation of reactive oxygen species which have damaging effects due to the oxidation of DNA, lipids or proteins. These conditions which are described as oxidative stress are often the result of tissue injuries. A pathophysiologically relevant role for reactive oxygen species has been suggested in various neurodegenerative diseases and stroke. Oxidative stress has also been linked to the development of atherosclerosis which requires in its early stages the oxidation of LDL particles for the formation of atherosclerotic plaques. Anti-oxidants have been shown to be able to treat and prevent various diseases to some degree. There are a variety of experimental drugs which can be used as anti-oxidants. Also, several endogenous anti-oxidants can be added to the nutrition like ascorbic acid, lipoic acid, carotenes, α -tocopherol and others. Also, fruits and vegetables are rich sources of natural anti-oxidants like polyphenoles (e.g. resveratrol) and anthocyanins.

- ▶ P450 Mono-oxygenase System
- ▶ Reactive Oxygen Species
- ▶ Oxidative Stress
- ▶ Vitamin C
- ▶ Vitamin E

Antiparasitic Drugs

Antiparasitic drugs are used for the treatment of parasitic infections caused by pathogenic protozoa or helminths (worms).

- ▶ Antihelminthic Drugs
- ▶ Antiprotozoal Drugs

Anti-Parkinson Drugs

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Synonyms

Antiparkinsonian drugs

Definition

Parkinsonism is a clinical syndrome comprising ▶bradykinesia, muscular ▶rigidity, ▶resting tremor, and impairment of postural balance. The pathological hallmark of Parkinson's disease is a loss of more than 60–70% of pigmented dopaminergic neurons of the substantia nigra pars compacta with the appearance of intracellular inclusions known as ▶Lewy bodies. Without treatment, idiopathic Parkinson's disease progresses over 5–10 years to a rigid, akinetic state, leading to complications of immobility, e.g., pneumonia and pulmonary embolism. The distinction between Parkinson's disease and other causes of parkinsonism is important because parkinsonism arising from other causes is usually more refractory to treatment with antiparkinsonian drugs.

Mechanism of Action

Pathophysiology

The primary deficit in Parkinson's disease is a loss of dopaminergic neurons in the substantia nigra pars compacta and a corresponding loss of dopaminergic innervation of the caudate nucleus and the putamen (forming the striatum). This suggests that replacement of dopamine could restore function. Physiologically, dopamine is synthesized from tyrosine in terminals of nigrostriatal neurons by the sequential action of the enzymes tyrosine hydroxylase, yielding the intermediary L-dihydroxyphenylalanine (L-DOPA), and aromatic L-amino acid decarboxylase (the corresponding prodrug L-DOPA is the most effective agent in the treatment of Parkinson's disease, see below.). The subsequent uptake and storage of synthesized dopamine in vesicles is blocked by reserpine, an earlier antipsychotic drug and admixture to antihypertensive medicines, which is known to induce parkinsonism. Release of dopamine is triggered by depolarization leading to entry of Ca^{2+} and ▶exocytosis. The pre- and postsynaptic actions of dopamine are mediated by two types of dopamine receptors, both of which are seven-transmembrane-region receptors. The D₁-receptor family (consisting of D₁ and D₅ receptors) stimulates the synthesis of intracellular cAMP and phosphatidyl inositol hydrolysis, but

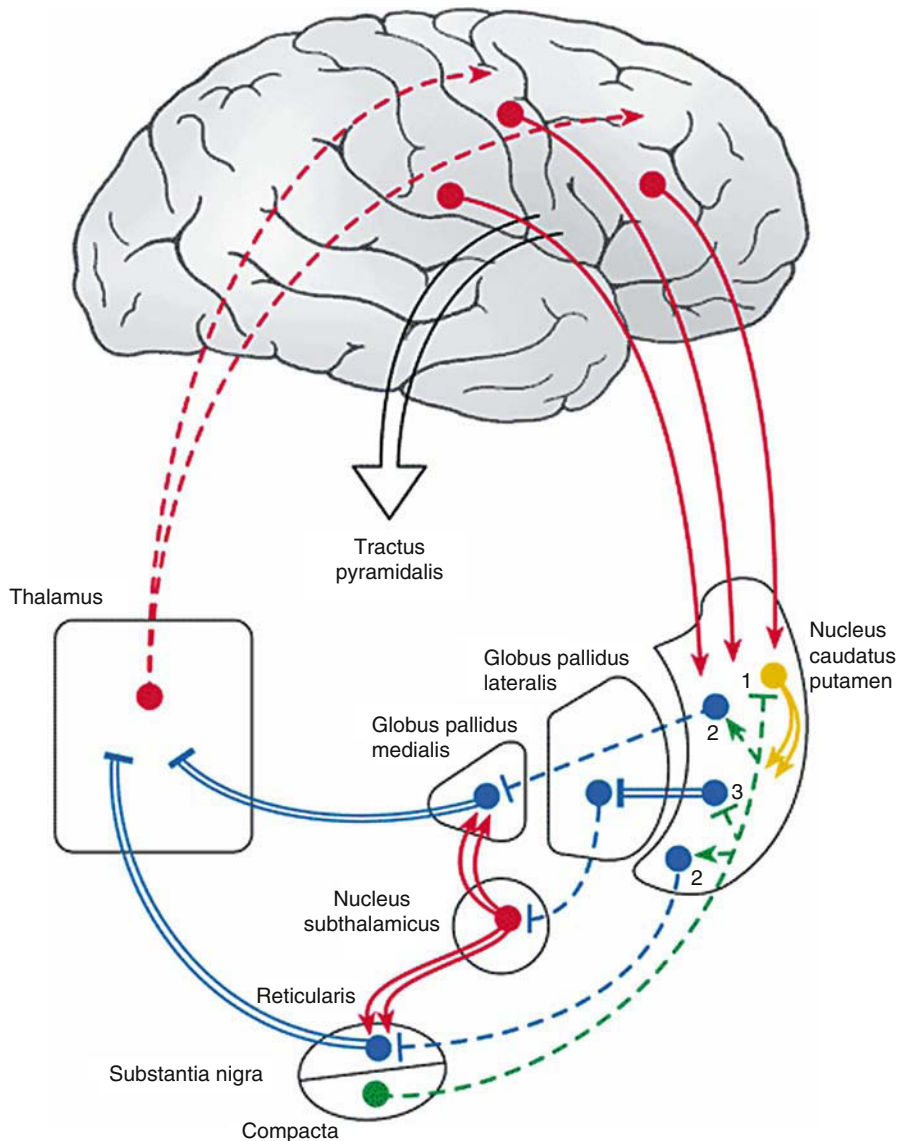
the D₂-receptor family (D₂, D₃ and D₄ receptors) inhibits cAMP synthesis and modulates K⁺ and Ca²⁺ channels. D₁ and D₂ proteins are abundant in the striatum; striatal D₃ expression is rather low. Most ►**antipsychotic drugs** block D₂ receptors and may lead to the adverse event of parkinsonism. As the disease progresses, neuron degeneration continues, involving other systems, including mesocortical dopaminergic cells, and noradrenergic, serotonergic, cholinergic, histaminergic, and peptidergic systems.

The following model of basal ganglia function accounts for the Parkinson syndrome as a result of diminished dopaminergic neurotransmission in the striatum (Fig. 1). The basal ganglia modulate the flow of information from the neocortex to the motoneurons in the spinal cord. The striatum receives excitatory glutamatergic input from the neocortex (red solid arrows). The majority of striatal neurons are projection neurons to other basal ganglia nuclei (blue GABAergic neurons) and a small subgroup is interneurons that interconnect neurons within the striatum (yellow cholinergic neurons). Nigrostriatal dopaminergic neurons (green) innervate GABAergic neurons (blue, 2, 3) and cholinergic interneurons (yellow, 1). The cholinergic interneurons mediate the dopaminergic control of corticostriatal long-term depression in GABAergic neurons. This long-term depression is due to a reduction of the muscarinic M₁ receptor tone at dendrites of GABAergic neurons, which receive glutamatergic input from neocortical, pyramidal neurons. Physiologically, the D₂ receptor-mediated reduction of the innervation of postsynaptic M₁ receptors leads to an enhanced Ca²⁺ influx into the dendrite of GABAergic neurons, resulting in enhanced endocannabinoid production, and a retrograde activation of presynaptic cannabinoid-1 receptors that diminish glutamate release. The outflow of the striatum proceeds as the direct and the indirect pathway. The direct pathway projects directly to the output stages of the basal ganglia, the substantia nigra pars reticulata and the globus pallidus medialis, which contain GABAergic neurons (blue). These in turn relay to the thalamus, which provides excitatory input to the neocortex (red broken arrows). Since two inhibitory GABAergic neurons are arranged successively, the stimulation of the direct pathway at the level of the striatum (by glutamatergic corticostriatal afferents or via 2) results in an increased excitatory outflow from the thalamus to the neocortex. The opposite effect, i.e., a decreased excitatory outflow from the thalamus, is the result when the stimulation of the first chain link of the direct pathway, GABAergic neurons in the striatum, is abolished.

This is the case when the excitatory D₁ receptors on these striatal GABAergic projection neurons are no longer activated since the transmitter dopamine is reduced (green broken arrows at 2). The indirect pathway is

composed of striatal GABAergic neurons (blue) that project to the globus pallidus lateralis (to blue GABAergic neurons). This inhibitory structure in turn innervates glutamatergic neurons of the subthalamic nucleus (red) to diminish the excitation of subthalamic neurons. The subthalamic nucleus provides excitatory glutamatergic outflow to the output stage, i.e., to GABAergic neurons (blue) of the substantia nigra pars reticulata and the globus pallidus medialis. Thus, the net effect of stimulating the indirect pathway at the level of the striatum is to reduce the excitatory outflow from the thalamus to the neocortex. Striatal neurons forming the indirect pathway express inhibitory D₂ receptors (3 in the Fig. 1), counteracting the excitation through glutamatergic corticostriatal afferents. Thus, dopamine released in the striatum reduces the activity of the indirect pathway through D₂ receptors, but increases the activity of the direct pathway through D₁ receptors. A reduced dopaminergic neurotransmission in the striatum (depicted as green broken line) ultimately reduces the thalamic excitation of the motor cortex. Note that the view of the striatofugal system as dual (direct/indirect) projections system has been questioned; it was suggested that GABAergic projection neurons project sequentially rather than in parallel to their major target areas, compatible with the repeatedly reported colocalisation of D₁ and D₂ receptors in these neurons.

What is the reason for the rather selective degeneration of nigrostriatal dopaminergic neurons in Parkinson's disease? Apart from their oxidative metabolism, leading to the production of reactive compounds as in every cell (hydrogen peroxide, superoxide anion radical), dopaminergic neurons seem to be additionally compromised by an extra accumulation of hydrogen peroxide due to the metabolic conversion of dopamine to 3,4-dihydroxyphenylacetaldehyde (DOPAL) plus hydrogen peroxide by the enzyme monoamine oxidase (MAO). In the presence of ferrous iron, hydrogen peroxide undergoes spontaneous conversion (Fenton reaction), forming a hydroxyl free radical, one of the most risky species of all reactive compounds. Levels of iron are high in the substantia nigra; it is, however, not clear whether the excess iron exists in a form, capable of participation in redox chemistry. In addition, the increase in iron occurs only in the advanced stages of Parkinson's disease, suggesting that this increase may be a secondary, rather than a primary initiating event. Despite this objection, hydroxyl-free radicals are generated from hydrogen peroxide without the catalytic help of ferrous iron in the presence of DOPAL: Thus, the either MAO product, DOPAL, is a cofactor in the generation of the hydroxyl radical from the other MAO product, hydrogen peroxide, which is also produced enzymatically by superoxide dismutase from the superoxide anion radical. Since MAO is located on the outer mitochondrial membrane, adjacent to the free radical



Anti-Parkinson Drugs. Figure 1 Extrapyramidal wiring diagram of the basal ganglia in Parkinson's disease. Arrow heads: activation; arrow beams: inhibition; solid lines: normal neurotransmission; double lines: increased neurotransmission; broken lines, diminished neurotransmission; red: glutamate excitatory; blue: GABA inhibitory; green: dopamine excitatory (D_1 receptors, 2) and inhibitory (D_2 receptors, 1, 3); yellow: acetylcholine. (from Feuerstein TJ. Antiparkinsonmittel, Pharmakotherapie des Morbus Parkinson. In: 5).

sensitive permeability transition pore, its products, including the hydroxyl free radical, may function as cell death messengers, leading to ►apoptosis. Apart from this local mechanism, reactive oxygen species can lead to DNA damage, peroxydation of membrane lipids, and neuronal death. Because parkinsonian brains are free of pathological signs of necrosis, apoptosis is the likely or predominant mechanism for the death of nigrostriatal dopamine neurons.

Emerging evidence suggests that dysfunction of the ubiquitin-proteasome system may be part of the pathophysiology of sporadic Parkinson's disease, especially

the association of parkin mutations with familial forms of the disease. Disease-linked mutations in parkin may cause defects in normal ubiquitin-proteasome system function with subsequent aberrant protein accumulation, resulting in proteolytic stress [1].

Neuromelanin, a dark colored pigment and product of the oxidative metabolism of dopamine, is found in the cytoplasm of dopaminergic neurons of the human substantia nigra pars compacta. Neuromelanin deposits increase with age, matching the age distribution of Parkinson's disease. In the absence of significant quantities of iron, neuromelanin can act as an antioxidant in

that it can interact with and inactivate free radicals. Neuromelanin functions as a redox polymer and may promote the formation of reactive oxygen free radicals, especially in the presence of iron that accumulates in neuromelanin. Thus, in the early stages of the disease, the iron-chelating properties of neuromelanin may act as a powerful protective mechanism, delaying symptom appearance and/or slowing disease progression. Once these protective mechanisms have been exhausted, the pathogenic mechanisms affecting cytoplasmic organelles, other than neuromelanin, destroy neuromelanin-harboring neurons, with consequent pouring out of neuromelanin granules. These, in turn, activate microglia, causing release of nitric oxide, interleukin-6, and tumor necrosis factor- α , thus becoming an important determinant of disease aggravation [2].

A genetic defect of complex I of the mitochondrial respiratory chain has been demonstrated specifically for the substantia nigra in Parkinson's disease. This finding matches the observation that the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes a Parkinson-like syndrome in humans, acts via inhibition of complex I by its neurotoxic metabolite 1-methyl-4-phenylpyridine (MPP⁺), thus destroying dopaminergic neurons in the substantia nigra. Despite this obvious specificity, the question arises whether dopaminergic neurons are more vulnerable to this mitochondrial deficit per se compared with other neurons and whether there is differential vulnerability to complex I inhibition within the dopaminergic substantia nigra population. In addition, which are the death transducers of mitochondrial dysfunction? Apart from increased accumulation of reactive oxygen species and their functional consequences (see above) due to defective mitochondria, dopaminergic neurons may express differentially regulated K_{ATP} channels in the plasma membrane. These channels respond to mitochondrial complex I inhibition. There may be a differential opening of K_{ATP} channels owing to a diminished ATP/ADP ratio in dopaminergic midbrain neurons. It has recently been suggested from mice models that K_{ATP} channels are causally linked to the differential degeneration of these dopaminergic neurons: K_{ATP} channel opening due to complex I inhibition was associated with hyperpolarization, functional silencing and, finally, cell death of substantia nigra dopamine neurons; cell death of dopaminergic neurons in the ventral tegmental area was absent, however, since complex I inhibition did not induce the opening of K_{ATP} channels in these neurons [3]. How should this K_{ATP} channel-induced neuronal silencing be linked with the death of dopamine neurons? This question remains open and the possibility that the opening of K_{ATP} channels is a futile effort of cellular self-protection due to another underlying degenerative mechanism should be considered. The fact that genetic inactivation of the

K_{ATP} channels under investigation resulted in selective rescue of substantia nigra dopaminergic neurons does not prove a deleterious, neurodegeneration-promoting role of K_{ATP} channels, but may simply mean that the dopamine neurons, lacking K_{ATP} channels, have developed other, undetected self-protection mechanisms.

Symptomatic Drug Therapy and Curative Treatments of the Future

While advances in the symptomatic drug therapy (summarized below) have certainly improved the lives of many Parkinson patients, the goal of current research is to develop treatments that can prevent, retard or reverse the death of dopaminergic neurons in the substantia nigra pars compacta (and of other neurons involved in the pathogenesis of Parkinson's disease not mentioned in this essay).

A large number of molecules have provided experimental evidence of neuroprotection in *in vitro* and *in vivo* models of Parkinson's disease and many of these putative neuroprotective substances are now the objects of clinical trials. Recently, a team of experts has identified potential neuroprotective agents to be tested in pilot studies [4]. Twelve compounds have been considered for clinical trials: caffeine, coenzyme Q 10, creatine, estrogen, GPI1485, GM-1 ganglioside, minocycline, nicotine, pramipexole, ropinirol, rasagiline, and selegiline (for individual discussion see [4]).

Clinical Use (Including Side Effects)

Oral L-DOPA is rapidly absorbed by the intestinal active transport system for aromatic amino acids, where dietary amino acids may act as competitors. The same is true at the corresponding aromatic amino acid carrier of the blood-brain barrier. L-DOPA is usually co-administered with a peripherally acting inhibitor of aromatic L-amino acid decarboxylase (benserazide, carbidopa) that prevents (dopamine-induced) nausea and vomiting, cardiac arrhythmias and orthostatic hypotension, and increases the fraction of L-DOPA that remains unmetabolized and available to cross the blood-brain barrier. The therapeutic and adverse effects of L-DOPA result from its intracerebral decarboxylation to dopamine. Entacapone is a selective inhibitor of catechol-O-methyltransferase, whose activity is primarily in the peripheral nervous system. Entacapone further increases the fraction of L-DOPA, crossing the blood-brain barrier, and thus prolongs its action and reduces fluctuations in response. In early Parkinson's disease, when some buffering capacity of remaining striatal dopaminergic nerve terminals is still present, the degree of motor improvement due to L-DOPA is highest (9). With time, however, the patient's motor state may fluctuate dramatically with each drug dose. Increasing the frequency of administration can

improve this situation, while increasing the L-DOPA dose may induce dyskinesias, i.e., excessive and abnormal involuntary movements. In view of the above-mentioned dopamine autotoxicity, might L-DOPA accelerate the disease progression? Although no convincing evidence for such an effect has yet been obtained, a pragmatic therapeutic approach may be appropriate, i.e., to use L-DOPA only when required by a functional impairment of the patient, not otherwise treatable.

Alternatives to L-DOPA are direct agonists of striatal dopamine receptors (e.g., pergolide, cabergoline) that are not metabolized in a manner that leads to increased free radical formation. Their use may reduce endogenous release of dopamine and the need for exogenous L-DOPA, possibly with the consequence of a delay in the progression of the disease. At present, however, there are no clinical data to support a neuroprotective effect of dopamine receptor agonists. In contrast to the prodrug L-DOPA, these agonists do not depend on the functional capacities of nigrostriatal nerve terminals, which may be advantageous in the late stages of Parkinson's disease where L-DOPA-induced fluctuations are frequent. In addition, clinically used dopamine agonists have durations of action substantially longer than L-DOPA. However, despite these pharmacokinetic advantages, the clinical efficacy of the currently available agonists that preferentially activate dopamine D₂ receptors is less than that of L-DOPA. Due to their peripheral activity, dopamine receptor agonists may cause orthostatic hypotension and nausea. Typical central adverse events in elderly patients are hallucinosis or confusion, similar to that observed with L-DOPA. In contrast to levodopa, D₂ receptor agonists impaired cognitive function in monkeys, most probably due to "tonic" activation of D₂ autoreceptors, and a similar deterioration was seen with pergolide in humans. In fact, activation of dopamine autoreceptors by drugs like pergolide and quinpirole depresses the neocortical release of dopamine in humans, i.e., phasic dopamine signaling and, thereby, learning performance. This could have deleterious effects especially in Parkinson patients, treated with D₂-receptor agonists, who in part are known to suffer in the first place from disease-related cognitive deficits.

The mode of action of selegiline, which slightly improves parkinsonian symptoms, is unclear. At clinically used doses, it inhibits the MAO-B isoenzyme whereas MAO-A prevails in dopaminergic terminals. Selegiline is metabolized to (–)-desmethyldiprenyl, which seems to be the active principle in its antiapoptotic effects in animal models, and further to (–)-amphetamine and (–)-methamphetamine. The (–)-amphetamines release biogenic amines, including dopamine from their storage sites in nerve terminals, although with less potency than their (+)-enantiomers.

This may partly explain the symptomatic relief seen with selegiline. Developmental drugs, structurally related to selegiline, which exhibit virtually no MAO-B or MAO-A inhibiting properties, and which are not further metabolized to amphetamines, show neurorescuing properties that are qualitatively similar, but obtained with about 100-fold more potency, compared to selegiline. Glyceraldehyde-3-phosphate dehydrogenase, a glycolytic enzyme with multiple other functions, including an involvement in apoptosis, seems to be the molecular target for these neuroprotective selegiline-related drugs of the future.

Adenosine A_{2A} receptors are localized to the indirect striatal output function and control motor behavior. Istradefylline is a novel adenosine A_{2A} receptor antagonist, which demonstrated a clinically meaningful reduction in motor fluctuations in L-DOPA-treated patients with established motor complications, and is safe and well tolerated.

Antagonists of muscarinic acetylcholine receptors had widely been used since 1860 for the treatment of Parkinson's disease, prior to the discovery of L-DOPA. They block receptors that mediate the response to striatal cholinergic interneurons. The antiparkinsonian effects of drugs like benztropine, trihexyphenidyl and biperiden are moderate; the resting tremor may sometimes respond in a favorable manner. The adverse effects, e.g., constipation, urinary retention, and mental confusion, may be troublesome, especially in the elderly.

Low affinity use-dependent NMDA receptor antagonists meet the criteria for safe administration into patients. Drugs like amantadine and memantine have modest effects on Parkinson's disease and are used as initial therapy or as adjunct to L-DOPA. Their adverse effects include dizziness, lethargy and sleep disturbance.

- Dopamine System
- Monoamine Oxidases

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α -2 Antiplasmin

α -2 antiplasmin, a naturally occurring inhibitor of fibrinolysis, is a single chain glycoprotein that forms a stable, inactive complex within plasmin and thereby prevents plasmin's activity.

►Coagulation/Thrombosis

Antiplatelet Drugs

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Synonyms

Platelet inhibitors; Platelet aggregation inhibitors

Definition

Platelets play a central role in ►primary hemostasis. They are also important in pathological processes leading to ►thrombosis. Antiplatelet drugs are primarily directed against platelets and inhibit platelet activation by a number of different mechanisms. They are used for the prevention and treatment of thrombotic processes, especially in the arterial vascular system.

Mechanism of Action

Antiplatelet therapy is an important means in the prevention and treatment of thromboembolic artery occlusions in cardiovascular diseases. Platelets are discoid cell fragments, derived from megakaryocytes in the bone marrow that circulate freely in the blood. Under normal conditions they neither adhere to each other nor to other cellular surfaces. However, when blood vessels are damaged at their luminal side, platelets adhere to the exposed subendothelium. Adherent platelets release various factors (see below) that activate other nearby platelets resulting in the recruitment of more platelets at the site of vascular injury. The rapid formation of a "platelet plug" at sites of vascular injury is the main mechanism of primary hemostasis. This is followed by a strengthening of the primary thrombus due to the formation of fibrin fibrils by the coagulation cascade. Platelets also play an important role in pathological conditions since they can become activated

on ruptured ►atherosclerotic plaques or in regions of disturbed blood flow. This in turn leads to thromboembolic complications that underlie common diseases such as myocardial infarction or thrombotic stroke.

Mechanisms of Platelet Activation

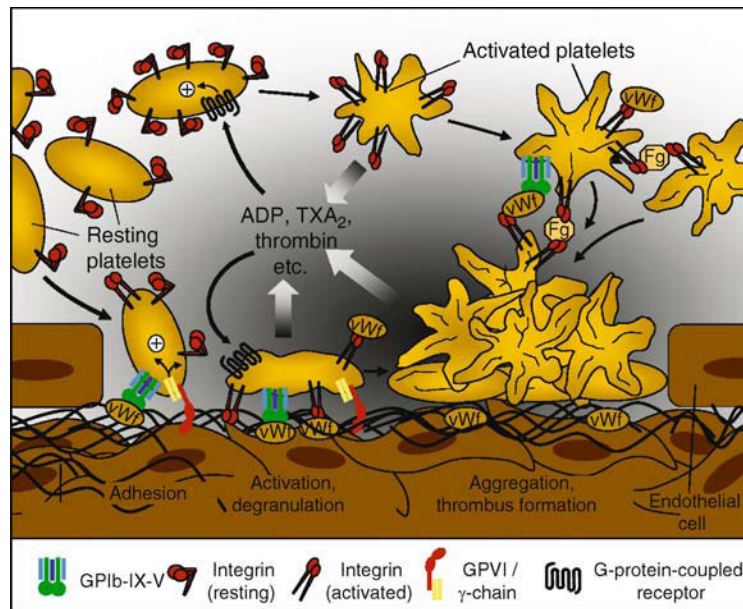
During the first phase of platelet activation, platelets adhere to extracellular matrix proteins of the subendothelium (see Fig. 1). Platelet adhesion is initially mediated by ►von Willebrand factor (vWf) which after binding to subendothelial collagen changes its conformation and interacts with the platelet receptor complex glycoprotein Ib-IX-V (GPIb-IX-V). This interaction brings platelets in contact with the subendothelium but does not result in a stable interaction. A stable adhesion of platelets is induced by the extracellular matrix protein ►collagen which via the platelet-specific receptor glycoprotein VI (GPVI) leads to an activation of platelets. This in turn results in the activation of several ►integrins like integrin α 2 β 1 and α IIb β 3 which then mediate the firm adhesion of platelets to collagen, fibronectin, or laminin exposed at the subendothelial surface.

The formation of a platelet aggregate requires the recruitment of additional platelets from the blood stream to the injured vessel wall. This process is executed through a variety of diffusible mediators which act through ►G-protein-coupled receptors. The main mediators involved in this process are adenosine diphosphate (ADP), thromboxane A₂ (TXA₂), and thrombin (factor IIa). These mediators of the second phase of platelet activation are formed in different ways. While ADP is secreted from platelets by exocytosis, the release of TXA₂ follows its new formation in activated platelets. Thrombin can be formed on the surface of activated platelets (see Fig. 2).

Initially, activated platelets change their shape, an event immediately followed by the secretion of platelet granule contents (including ADP, ►fibrinogen, and serotonin) as well as by platelet aggregation. Aggregation of platelets is mediated by fibrinogen or vWf. They connect platelets by bridging complexes of glycoprotein IIb/IIIa (integrin α IIb β 3) on adjacent platelets, forming a platelet aggregate. Each platelet contains about 50,000–80,000 glycoprotein IIb/IIIa (GPIIb/IIIa) molecules on its surface. In order to bind fibrinogen and vWf, GPIIb/IIIa has to be converted from low affinity/avidity state to a high affinity/avidity state by a process described as inside-out signaling that is initiated during platelet activation (Fig. 2).

Acetylsalicylic Acid (Aspirin)

TXA₂ is produced by activated platelets via the sequential conversion of arachidonic acid by phospholipase A₂, ►cyclooxygenase-1 (COX-1), and thromboxane synthase. Similar to ADP, TXA₂ acts as a



Antiplatelet Drugs. Figure 1 Platelet adhesion, activation, aggregation and thrombus formation on subendothelial surface at an injured blood vessel. After an injury of the vascular endothelium, the processes of primary hemostasis (platelet activation) as well as of secondary hemostasis (fibrin formation via the coagulation cascade) are triggered by a variety of stimuli. Shown are only the mechanisms of primary hemostasis. Platelets adhere via their receptor GPIb-IX-V and GPVI mediated by von-Willebrand factor (vWf) or binding of extracellular matrix (especially collagen) to the subendothelium. Binding of collagen to GPVI initiates platelet activation resulting in integrin activation and the formation of a variety of diffusible mediators like ADP, thromboxane A₂ (TXA₂) as well as thrombin. These mediators initiate the second phase of platelet thrombus formation by recruiting platelets from the blood stream into a growing platelet aggregate. The crosslinking of platelets primarily involves the activated integrin αIIbβ3 (GPIIb/IIIa) which can bind the bivalent ligands vWf and fibrinogen (Fb) resulting in the crosslinking of platelets.

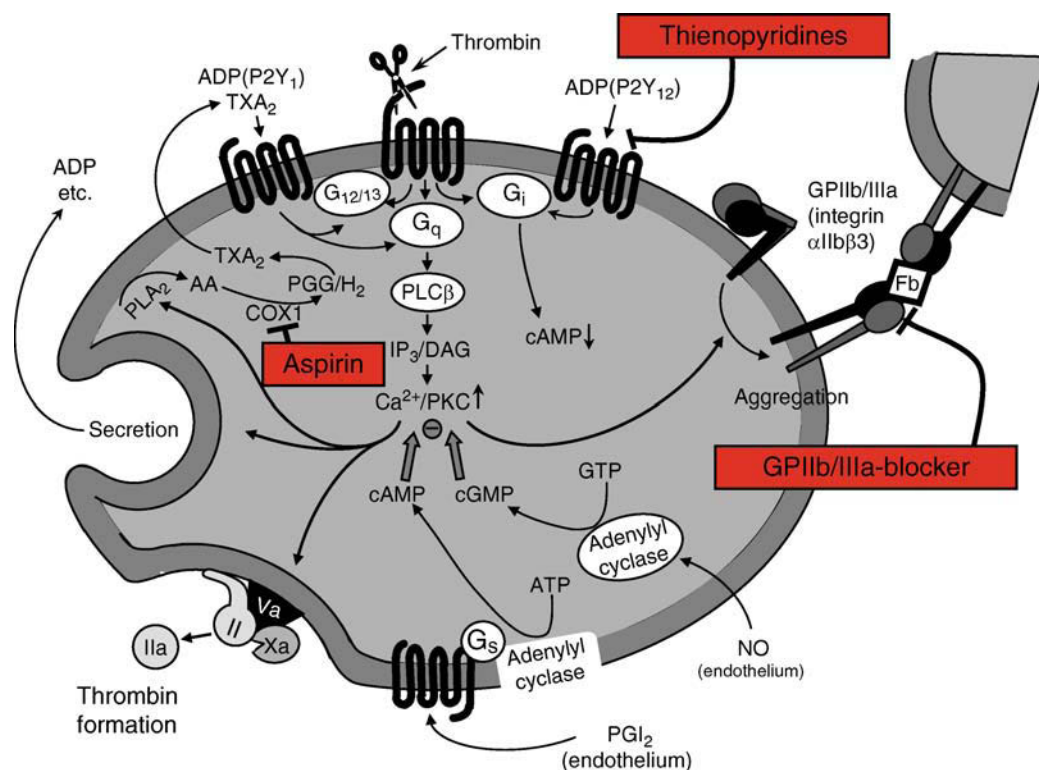
positive feedback mediator. In vascular endothelial cells, COX-1 is involved in the generation of prostacyclin (PGL₂), which inhibits platelet activation and leads to vasodilation. Low doses of acetylsalicylic acid (aspirin) have an antiplatelet effect by inhibiting the TXA₂ production by irreversibly acetylating COX-1 at serine-530 close to the active site of the enzyme which interferes with the binding of the substrate arachidonic acid to the enzyme. This results in impaired platelet function for the rest of its lifespan (7–10 days). Anucleated platelets, in contrast to nucleated cells, are unable to *de novo* synthesize COX-1. The aspirin doses required for this antiplatelet effect are therefore considerably lower than those necessary to achieve inhibition of prostacyclin formation in endothelial cells or analgetic and antipyretic effects elsewhere in the body. Following oral administration of aspirin, platelets are exposed to a relatively high concentration of aspirin in the portal blood. This may further contribute to the relatively high sensitivity of platelets toward the action of aspirin. Most other tissues are partly protected from irreversible COX-1 inhibition by presystemic metabolism of aspirin to salicylate through esterases in the liver.

Thienopyridines

ADP is released from activated platelets by the secretion of dense granules and acts through at least three receptors. These are the ionotropic purinoceptor 2X₁ (P2X₁) and two G-protein-coupled receptors, the G_q-coupled purinoceptor 2Y₁ (P2Y₁), and the G_i-coupled P2Y₁₂ receptor. The latter has also been termed P2T_{AC} or P2_{cyc} and is targeted by a group of antiplatelet agents – the thienopyridines – such as ticlopidine and clopidogrel. To become activated, ticlopidine and clopidogrel require biotransformation by the hepatic CYP3A4 enzyme into active metabolites. The active metabolites irreversibly modify the P2Y₁₂ receptor. Due to the requirement of the formation of active metabolites, thienopyridines have a delayed onset of action. Similar to the antiplatelet effects of aspirin, the effects of thienopyridines are long-lasting due to the irreversible inhibition of the P2Y₁₂ receptor.

GPIIb/IIIa (Integrin-IIbβ3) Inhibitors

Most antiplatelet drugs only partially inhibit platelet activation. In contrast, blockers of GPIIb/IIIa interfere at the end of the pathway common to platelet aggregation. They prevent fibrinogen and vWf from



Antiplatelet Drugs. Figure 2 Mechanisms of platelet activation, together with sites of drug action. Most platelet activators function directly or indirectly through G-protein-coupled receptors and induce several intracellular signalling pathways that eventually lead to secretion of granule contents, change of shape, inside-out activation of GPIIb/IIIa (integrin α IIb β 3), the exposure of factor Va and subsequent formation of the prothrombinase complex which forms thrombin (factor IIa) as well as the activation of phospholipase A₂ (PLA₂). Activation of GPIIb/IIIa allows fibrinogen (Fb) or vWf to cross bridge adjacent platelets. The main pathway that leads to platelet activation involves the G_q/phospholipase C- β (PLC- β)-mediated formation of inositol 1,4,5 trisphosphate (IP₃) and diacyl glycerol (DAG). This in turn results in the release of Ca²⁺ from intracellular stores and the activation of protein kinase C (PKC) isoforms. Major inhibitors of platelet activation are the endothelium-derived mediators nitric oxide (NO) and prostacyclin (PGI₂). While NO via activation of guanylyl cyclase increases cGMP levels, PGI₂ via activation of a G_s-coupled receptor increases the levels of cAMP. Both cyclic nucleotides inhibit via different mechanisms signaling processes involved in platelet activation. Aspirin blocks the conversion of arachidonic acid (AA) to prostaglandin G₂ and H₂ (PGG/H₂) by irreversibly inhibiting cyclo-oxygenase-1 (COX-1). Active metabolites of thienopyridines block ADP (P2Y₁₂)-receptors on platelets and GPIIb/IIIa-blockers interfere with fibrinogen- and vWf-mediated platelet aggregation. TXA₂ stands for thromboxane A₂.

binding to activated GPIIb/IIIa and can therefore completely inhibit platelet aggregation. The first GPIIb/IIIa antagonist developed was a hybrid human/murine monoclonal antibody. Its Fab fragment, termed abciximab, is clinically used and functions in a noncompetitive manner. An alternative approach to block GPIIb/IIIa involves the use of peptides that mimic short protein sequences of fibrinogen or vWf. Several peptides (e.g. the cyclic heptapeptide eptifibatide) or nonpeptidic, low molecular weight compounds (e.g. tirofiban, lamifiban) have been developed and function as competitive antagonists (Table 1).

Others

The proteolytic enzyme thrombin is known to play a crucial role in the overall thrombotic event leading to

both, arterial and venous thrombosis by transforming fibrinogen into fibrin and by serving as a direct platelet activator. Thrombin exerts its effects on platelets via G-protein-coupled protease-activated receptors (PAR-1 and PAR-4 in human platelets). Thrombin-dependent receptor activation is achieved by cleaving an N-terminal extracellular peptide. Exposure of the newly generated N-terminal region functions as a tethered ligand for the receptor. Substances that directly bind to thrombin have been developed. The 65 amino acid long protein hirudin, originally isolated from the medical leech, *Hirudo medicinalis*, as well as related analogs have been recombinantly produced. They bind with the stoichiometry of 1:1 to thrombin and prevent its proteolytic action on fibrinogen as well as its binding to and the activation of PAR. Since they primarily act by

Antiplatelet Drugs. Table 1 Pharmacological properties of GP IIb/IIIa inhibitors

	Abciximab	Eptifibatide	Tirofiban
Molecular weight (Da)	50,000	800	500
Integrin selectivity	α IIb β 3; α V β 3	α IIb β 3	α IIb β 3
Affinity for α IIb β 3 (K_D ; nmol/l)	5	120	15
Plasma half life	0.5 h	2–2.5 h	2 h
Duration of action	12–24 h	2–2.5 h	2 h
Elimination	Proteolysis/renal	Mainly renal	Mainly renal

inhibiting thrombin-dependent fibrin formation, they are generally classified as ►**anticoagulants**.

Since platelets are the major source of TXA₂ production and action, inhibitors of thromboxane synthase and TXA₂ receptor (TP) antagonists are being developed. TXA₂ synthesis inhibitors may have some disadvantages as they lead to the accumulation of cyclic endoperoxides (e.g. PGH₂) that are themselves agonists at the TXA₂ receptor.

The major physiological inhibitors of platelet activation are endothelium-derived mediators like prostacyclin (PGI₂) which via a G_s-coupled receptor activates the formation of cAMP formation by adenylyl cyclase as well as nitric oxide (NO) which stimulates the formation of cGMP by activating guanylyl cyclase. NO-generating drugs like organic nitrates lead to platelet inhibition, however, their main effect is on the vascular smooth muscle. Dipyridamole can inhibit the degradation of cAMP by inhibition of cyclic nucleotide phosphodiesterase and has been used as an antiplatelet agent. However, its clinical usefulness is not clear.

Clinical Use

Due to the pivotal role of platelets in thrombus formation, especially in the arterial system, inhibition of platelet function has become a central pharmacological approach. Antiplatelet drugs are given in order to prevent and treat thromboembolic diseases such as coronary heart disease, peripheral and cerebrovascular disease. They have also revolutionized the procedures of invasive coronary interventions as they reduce the risk of restenosis and thrombosis.

Aspirin leads to maximal anti thrombotic effects at doses much lower than required for other actions of the drug. Clinical trials have demonstrated that aspirin is maximally effective as an antithrombotic drug at daily doses of 75–160 mg. Higher doses have no advantage but increase the frequency of side effects, especially bleeding and upper gastrointestinal symptoms. Despite the development of various other compounds, aspirin has remained the gold standard for antiplatelet drugs due to its relative safety and extremely low cost. Several studies have demonstrated a beneficial role for aspirin as an adjunctive therapy in unstable angina and acute myocardial infarction. Mortality and disease

progression were significantly reduced by low dose aspirin treatment. Patients with a history of arterial thromboembolism including myocardial infarction, stroke, transient ischemic attack, or unstable angina were shown to benefit from low dose aspirin treatment in several trials. The overall rate of mortality, as well as the occurrence of further vascular events was reduced in these patients. The results of these studies led to the recommendation to use aspirin for secondary prevention of arterial thromboembolism. However, aspirin is not generally recommended for primary prevention of arterial thromboembolism. A possible beneficial effect, such as a decreased risk of nonfatal myocardial infarction, may outweigh the risk of hemorrhagic complications only in a population already at high risk of cardiovascular diseases but not in a population of average health. Aspirin may also be beneficial as a prophylactic agent to reduce the risk of deep venous thrombosis and pulmonary embolism. However, the effectiveness compared to existing therapies remains to be determined; anticoagulants are still the mainstay of treatment in these conditions.

Thienopyridines are principally suited to treat conditions that respond to aspirin. Ticlopidin, but not clopidogrel, can lead to fatal neutropenia. Gastrointestinal problems and skin rashes can occur with both drugs but are more frequently seen when ticlopidine is used. In various trials, clopidogrel has been shown to be safe and similarly effective as aspirin. In patients at high risk from cerebrovascular events, thienopyridines seem to be somewhat more effective than aspirin in preventing serious vascular complications. Thienopyridines may be used instead of aspirin when the latter is not tolerated. However, aspirin still remains the first choice in most cases due to its low cost, relative safety and well documented efficacy. Studies are under way to test whether clopidogrel, given together with aspirin has advantages under certain clinical conditions.

GPIIb/IIIa antagonists have to be administered parenterally. They are currently used prophylactically during intracoronary interventions such as percutaneous transluminal revascularization with balloon angioplasty or intracoronary stenting, as well as to treat acute coronary syndromes like unstable angina and acute myocardial infarction. The main complications

are bleeding and thrombocytopenia. The bleeding risk appears to increase further with concomitant therapy with heparin at standard doses.

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Antiprogestins

Antiprogestins are progesterone receptor antagonists such as mifepristone (RU 38486), ORG 31710, ZK 137 316, ZK 230 211, ZK98299 (Onapristone).

- Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
- Selective Sex Steroid Receptor Modulators

Antiproliferative Agents

- Cancer, Molecular Mechanism of Therapy
- Antineoplastic Agents
- Alkylating Agents
- Antimetabolites

Antiprotozoal Drugs

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Synonyms

Protocidal drugs; Antiprotozoan chemotherapeutics

Definition

Protozoa are unicellular eukaryotes and a subregnum of the animal kingdom. Some protozoa exhibit a parasitic life style and are pathogenic to humans, animals and plants. An estimated 1.5 billion people suffer from protozoal infections, with malaria (*Plasmodium* spp.) alone causing an estimated 500 million clinical cases each year. Other examples of important human infectious diseases with protozoan etiology are as follows: toxoplasmosis (*Toxoplasma gondii*), African sleeping sickness (*Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*), Chagas disease (*Trypanosoma cruzi*); visceral, mucocutaneous, and cutaneous leishmaniasis (*Leishmania* spp.), amoebic colitis and liver abscess (*Entamoeba histolytica*) and lambic enteritis (*Giardia lamblia*). Although there is a huge demand for antiprotozoal drugs, particularly drugs affordable by people living in developed countries, the incentives to develop such drugs are low. Accordingly, pharmaceutical industry has invested in other areas, such as cardiovascular, where revenues are higher (Table 1). Only recently have private–public partnerships and international institutions, such as Drugs for Neglected Disease Initiative (► <http://www.dndi.org/>), the Medicines for Malaria Venture (► http://www.mmv.org/papes/page_main.htm), the Institute for One World Health (► <http://www.oneworldhealth.org/>), the WHO Tropical Disease Research Programme (► <http://www.who.int/tdr/>), tried to fill this gap. In the following chapter, important antiprotozoal drugs (Fig. 1), including their modes of action, will be discussed (Table 1).

Antimalarial Drugs

Four different protozoa of the genus *Plasmodium* – *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* – can cause malaria. *P. falciparum* is the most virulent, being responsible for virtually all fatal malaria cases. Humans are infected by a feeding female Anopheles mosquito (Fig. 2). The clinical symptoms of malaria are associated with the development of the parasite within human red blood cells, while the liver stages remain asymptomatic. The following drugs (in alphabetical order) are currently in use for the treatment of malaria [5].

Mechanism of Action

Amodiaquine, a Mannich base 4-aminoquinoline, eliminates blood stage parasites. Its mode of action is similar to that of chloroquine (see below) and there is some cross-resistance.

Artemisinin and its derivatives, artesunate and arthemether, kill both asexual and sexual blood stages (Fig. 2). However, artemisinins are quickly eliminated from the body, resulting in parasite recrudescence, and are therefore combined with schizontocides that have a longer biological half-life, such as amodiaquine,

Antiprotozoal Drugs. Table 1 New compounds registered between 1975 and 1999 (with permission Trouiller et al., 2003 Lancet 359: 2188–2194)

Speciality	New compounds (1975–1999)	Proportion (%) of worldwide sales
	n (% of total)	
Cardiovascular	179 (12.8)	19.8
Antiinfectious (including)	224 (16.1)	10.3
Antiparasitic	26 (1.9)	1.5
HIV/AIDS	3 (0.2)	0.2
TB	13 (0.9)	0.2
Tropical diseases	4 (0.3)	0.1
Malaria		

lumefantrine, mefloquine and sulfadoxine/pyrimethamine. The artemisinins have been shown to be potent inhibitors of a plasmodial endoplasmic Ca^{2+} ATPase. A single-point mutation within the Ca^{2+} ATPase can abolish inhibition by artemisinin, although resistance to artemisinins has not been reported in the field. Artemisinin is a sesquiterpene lactone extracted from the leaves of *Artemisia annua*. Also known as qinghaosu, artemisinin has been used in China for the treatment of fever for more than 1,000 years.

Atovaquone, a hydroxynaphthoquinone, selectively inhibits the respiratory chain of protozoan mitochondria at the cytochrome bc1 complex (complex III) by mimicking the natural substrate, ubiquinone. Inhibition of cytochrome bc1 disrupts the mitochondrial electron transfer chain and leads to a breakdown of the mitochondrial membrane potential. Atovaquone is effective against all parasite stages in humans, including the liver stages.

Chloroquine, a 4-aminoquinoline, targets the intra-erythrocytic stages of malarial parasites (Fig. 2). Its mode of action is intricately linked with the plasmodial heme metabolism (Fig. 3). During development within erythrocytes, *Plasmodia* feed on the host cell's haemoglobin, which is digested within an acidic food vacuole. Heme released during haemoglobin proteolysis is highly cytotoxic and perforates cellular membranes. Malarial parasites detoxify heme through biomineralization within their food vacuoles to insoluble and inert ►haemozoin (malaria pigment). Chloroquine, which accumulates in the food vacuole, prevents heme biomineralization by forming complexes with heme, resulting in a build-up of toxic heme–chloroquine complexes that have an even higher affinity for membranes than heme alone and eventually destroy the parasite's cellular membranes (Fig. 3). Chloroquine resistance is linked with polymorphisms within a food vacuolar transporter that, according to current models, mediates efflux of the drug from the food vacuole.

Dapsone, an aromatic sulfone, is administered in combination with a proguanil derivative. Dapsone

inhibits the plasmodial dihydropteroate synthase (DHPS) (Fig. 4).

Tetracycline and its derivative doxycycline are antibiotics widely used in the treatment of bacterial infections. They also exert an antimalarial activity. Tetracyclines inhibit the binding of aminoacyl-tRNA to the ribosome during protein synthesis.

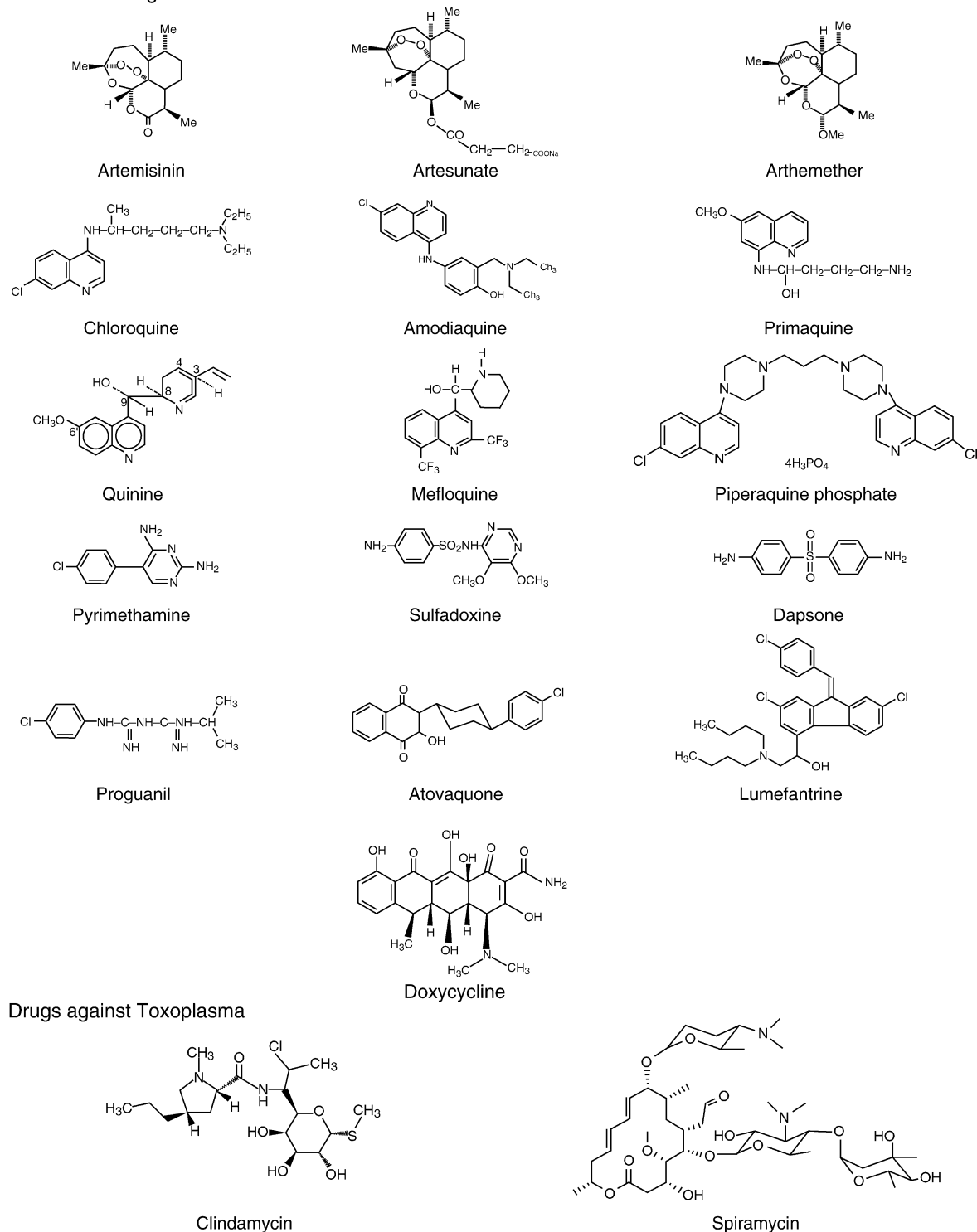
Piperaquine, a bisquinoline, is a rapid acting blood schizonticide. The mode of action is unknown.

Primaquine, an 8-aminoquinoline, eradicates the dormant stages (hypnozoites) of *P. vivax* and *P. ovale* from the liver. Its mode of action remains obscure.

Proguanil appears to have a dual activity. Part of it is metabolized to cycloguanil, which subsequently inhibits the protozoan dihydrofolate reductase/thymidylate synthase (DHFR/TS) (Fig. 4). In addition, the native form, proguanil itself, exerts a potent antimalarial activity, especially in combination with other antimalarial drugs. The target of proguanil is unknown.

Pyrimethamine, cycloguanil and sulfadoxine (sulfadiazine) are folate antagonists that interfere with the folic acid biosynthesis pathway in malarial parasites and other protozoa, including *T. gondii* (Fig. 4). Folate is an essential precursor of the pyrimidine deoxythymidintriphosphate (dTTP) and the amino acids serine and methionine. Both protozoa and mammalian cells require folate for DNA and protein synthesis. However, protozoa can either synthesize dihydrofolate de novo or salvage folate precursors, whereas mammalian cells have no de novo dihydrofolate synthesis and must rely on dietary sources. By acting as an analogue of *p*-aminobenzoic acid, sulfadoxine (sulfadiazine) inhibits the DHPS, which then fails to convert dihydropteroate to hydroxymethyldihydropterin, resulting in a lack of dihydrofolate in the parasite. This mechanism does not affect the mammalian cells. Pyrimethamine and cycloguanil, the active metabolite of proguanil, act further down in the folic acid pathway by inhibiting the DHFR/TS enzyme complex. In mammalian cells, the DHFR and the TS are two independent

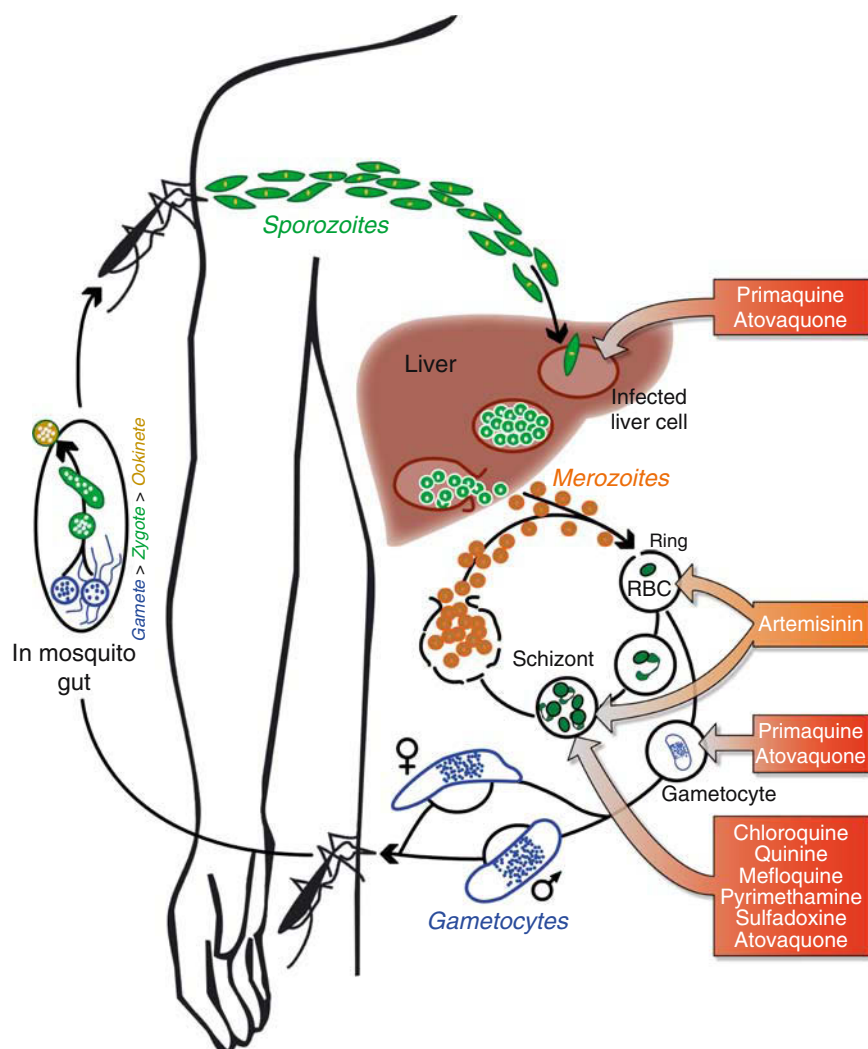
Antimalarial drugs



Antiprotozoal Drugs. Figure 1 Chemical structure of relevant antiprotozoal drugs.

Quinine, an arylaminoalcohol, was the first antimalarial known to the Western world. It was originally produced from the bark of the cinchona tree and distributed as a powdery substance, which became

known as Jesuits powder. Several potent antimalarials are derived from quinine, including lumefantrine, mefloquine, halofantrine and quinidine, the dextrarotatory diastereoisomer of quinine. All arylaminoalcohols



Antiprotozoal Drugs. Figure 2 Life cycle of malarial parasites and site of action of different antimalarial drugs.

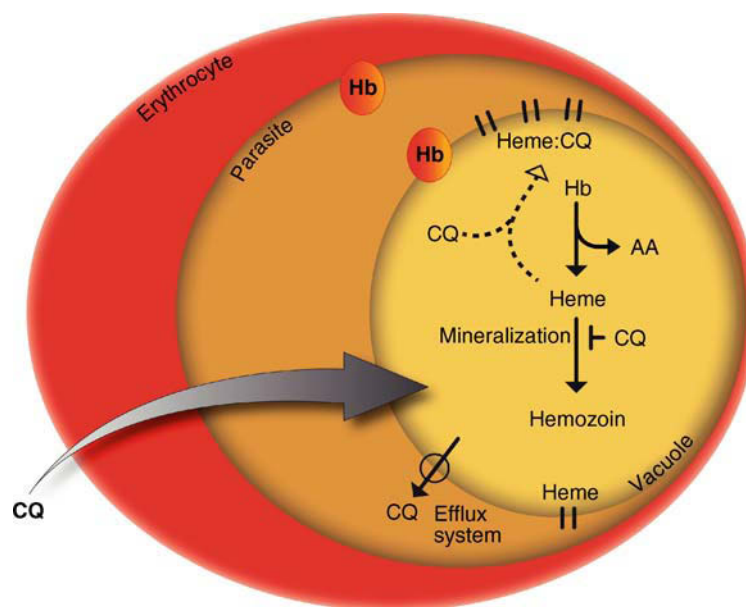
Malaria is caused by protozoan parasites of the genus *Plasmodium*. Infected *Anopheles* mosquitoes transmitted the parasite to humans during blood feeding. The infective stages are the sporozoites, which invade liver cells where they replicate to form merozoites. Upon rupture of the infected hepatocyte, merozoites are released into the blood stream where they infect erythrocytes. Within the erythrocyte, the parasites develop from ring stages to trophozoites, and then to schizonts. When the infected erythrocyte finally ruptures, merozoites are released, which again invade erythrocytes. Some intraerythrocytic ring stages develop to sexual stages (gametocytes). Gametocytes are taken up by feeding *Anophelines*. Within the mosquito, the parasites develop into gametes, zygotes, ookinets and finally sporozoites. Primaquine and atovaquone are effective against liver forms of all *Plasmodia* including dormant stages of *P. vivax* and *P. ovale*. Primaquine also acts against gametocytes. Artemisinin destroys intraerythrocytic ring stage parasites and schizonts. The so-called schizonticidal drugs, chloroquine, quinine, mefloquine, pyrimethamine and sulfadoxine, act against intraerythrocytic schizonts.

are believed to kill asexual blood stages by inhibiting heme detoxification in the parasite's food vacuole (Fig. 3), although the mechanism of action is not well-understood.

Clinical Use

Malaria treatment has relied on a small number of chemically related drugs of the quinoline and the

antifolate group for more than 4 decades [5]. Artemisinin derivatives have been added only recently as a third group [1]. Various degrees of resistance and geographic distributions of resistance phenotypes have developed for all classes of antimalarials, except the artemisinin derivatives, and is limiting their use [1, 5]. Chloroquine now fails in the treatment of falciparum malaria almost everywhere [5]. To prevent, or at least slow down, the



Antiprotozoal Drugs. Figure 3 The antimalarial activity of chloroquine. Chloroquine's mode of action is associated with heme detoxification. During intraerythrocytic development, *P. falciparum* degrades hemoglobin down to amino acids (AA) in its acidic food vacuole. Heme released from hemoglobin (Hb) is toxic and destroys cellular membranes unless it is converted to an inert biomineral, termed hemozoin or malaria pigment. Chloroquine (CQ) binds to heme, thereby preventing biomineralization of heme. The build-up of membrane-lytic heme/chloroquine complexes kill the parasite. Chloroquine resistant parasites have acquired an efflux system that expels the drug from the food vacuole.

emergence of resistance, compounds with different modes of action are being combined. The choice of drug, the route of drug application, and the dose regimen depend on the type of infection and the severity of the disease. The primary objective of treating uncomplicated malaria is to cure the infection, whereas in severe malaria it is to prevent death.

Severe Malaria

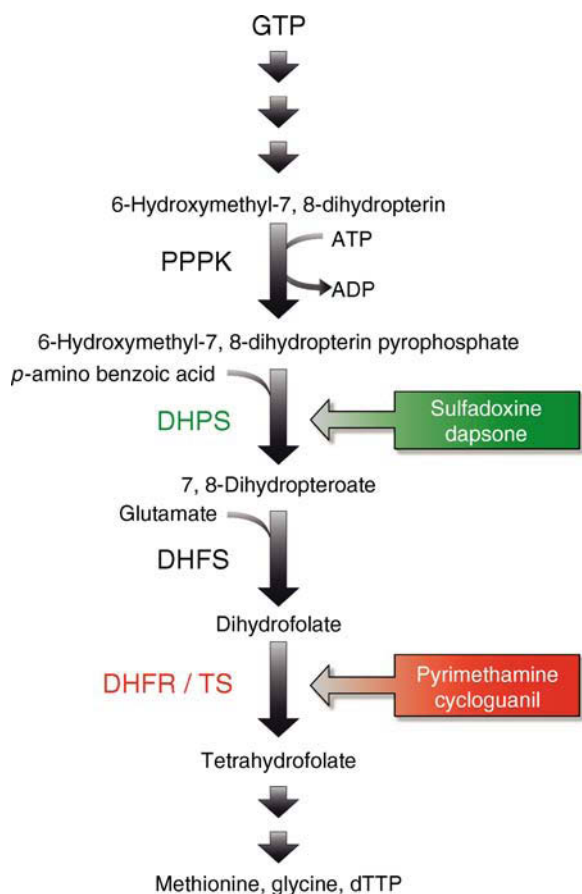
In severe malaria, rapid clearance of parasites is vital. This is achieved using an antimalarial compound that rapidly kills the parasites [5]. The compound has to be given intravenously as soon as possible with a loading dose at the start. A loading dose substantially cuts down the time by which therapeutic levels are achieved. Two classes of currently available drugs fulfill these requirements, the quinolines quinine and quinidine and the artemisinin derivatives artesunate, artemether, and artemotil. The pharmacokinetic properties of artesunate are superior to those of artemether and artemotil. It is water-soluble and can be given parenterally. Randomized trials in Southeast Asia, comparing artesunate with quinine, have clearly demonstrated the benefits of artesunate in reducing mortality rates by almost 35% as compared with quinine. Based on these results, artesunate has been suggested as the treatment of choice for severe malaria in adults. This

recommendation cannot yet be extended to children, particularly from high transmission settings. In African children, no significant difference in mortality rates were found when comparing artemether with quinine.

Uncomplicated Malaria

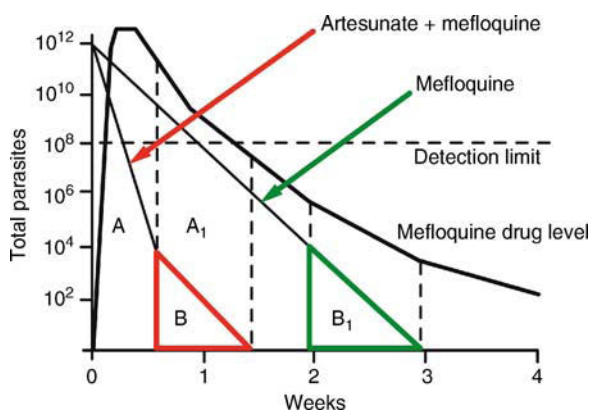
The treatment of uncomplicated malaria seeks to prevent recrudescence and, at the same time, tries to prevent the development of resistance. This is the rationale behind artemisinin-based combination therapies (ACT). Artemisinin and its derivatives (artesunate, artemether, artemotil, dihydroartemisinin) are clearing both parasitaemia and symptoms rapidly [1]. There is little difference in absorption and bioavailability amongst the different artemisinin derivatives. Due to the extraordinary high parasite clearance rate, artemisinin and its derivatives quickly reduce the total burden of parasites to very low levels. Thereafter, the eradication of the remaining parasites relies on the partner drug in ACTs. The partner drug needs to be effective and parasitocidal concentrations have to be sustained until all parasites have been killed. A slowly eliminated drug is ideal. The artemisinin derivatives are well tolerated with the exception of type 1 hypersensitivity reactions, which appear to be rare (1:3,000).

ACTs with amodiaquine, atovaquone-proguanil, chloroquine, clindamycin, doxycycline, lumefantrine,



Antiprotozoal Drugs. Figure 4 Mode of action of folate antagonists in protozoa. Protozoa are capable of *de novo* synthesis of dihydrofolate, a precursor of thymidine, serine and methionine. The 6-hydroxymethyl-7,8-dihydropterin pyrophosphate pyrophosphokinase (PPPK) and DHPS form one enzyme complex with two distinct active sites. The PPPK transforms GTP to 6-hydroxymethyl-7,8-dihydropterin and then to 6-hydroxymethyl-7,8-dihydropterin pyrophosphate. The product is linked to *p*-aminobenzoic acid (PABA) by DHPS, forming 7,8-dihydropteroate. Sulfadoxine exerts its activity by acting as a substrate analogue of PABA in this reaction. Glutamate is added to 7,8-dihydropteroate by dihydrofolate synthase (DHFS) resulting in dihydrofolate. Dihydrofolate reductase (DHFR) and thymidylate synthase (TS) form a single enzyme complex with two enzymatically active sites. The catalytic site responsible for DHFR activity converts dihydrofolate into tetrahydrofolate. Pyrimethamine and cycloguanil exert their antimalarial activity by inhibiting the protozoal DHFR. The site of DHFR/TS responsible for TS activity converts tetrahydrofolate into deoxythymidinotriphosphate (dTTP).

mefloquine, piperaquine, pyronaridine, proguanil–dapsone, sulfadoxine–pyrimethamine, and tetracycline have been evaluated in trials (Fig. 5) [1]. The following



Antiprotozoal Drugs. Figure 5 Artemisinin combination therapy (ACT): Adding a 3-days artesunate course to mefloquine clears the parasitaemia much more rapidly ($A_1 \rightarrow A$). The remaining parasites are exposed to higher mefloquine levels in ACT (B) compared to mefloquine monotherapy (B_1) (with permission White, 1997 *Antimicrob Agents Chemother* 41:1413–1422).

ACTs are currently recommended: artemether–lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, artesunate + sulfadoxine–pyrimethamine [1, 5]. In areas with amodiaquine and sulfadoxine–pyrimethamine resistance exceeding 20%, i.e., SE Asia, artesunate + mefloquine or artemether–lumefantrine should be used [1, 5].

Malaria Treatment and Chemoprophylaxis in Nonimmune People

Travelers treated for malaria after returning to nonendemic regions are treated along the same lines as above regarding uncomplicated and severe forms of the disease. Considerations with respect to development of resistance can be neglected. Monotherapy is fully justified and the following drugs are recommended: artemether–lumefantrine, atovaquone–proguanil, mefloquine, and quinine + doxycycline or clindamycin [5]. For the millions of nonimmune travelers to malaria-endemic areas, chemoprophylaxis (high-risk areas) and stand-by treatment (low-risk areas) are the corner stones of malaria prevention [5], along with exposition prophylaxis using repellent and impregnated bed-nets.

Intermittent Preventive Therapy in Pregnant Women and Infants

In endemic areas, chemoprophylaxis has been abandoned for a variety of reasons, mainly due to sustainability problems and the risk of contributing to the development of resistance. Intermittent preventive therapy (IPT), however, appears to be an alternative to protect pregnant women (IPT_p) and children during the first year of their life (IPT_i).

Antimalarial Drugs (*P. vivax*, *P. ovale*, *P. malariae*)

Resistance of *P. vivax* to chloroquine occurs, but is geographically limited. *P. vivax* and *P. ovale* produce hypnozoites, parasite stages in the liver that can produce multiple relapses. The drug of choice for blood stage infections to date remains chloroquine for all three species. To achieve radical cure of *P. vivax* and *P. ovale* infection, this must be followed by primaquine.

Antileishmanial Drugs

Leishmania is a disease complex caused by different species of *Leishmania*. The parasite, which is transmitted to humans by the bite of phlebotomine sandflies, multiplies within human macrophages. There are an estimated 1.5 million cases in approximately 88 countries each year, with 0.5 million patients suffering from the visceral leishmaniasis.

Mechanism of Action

Amphotericin B, is a polyene antibiotic, used in the therapy of systemic fungal infections. Its mode of action exploits differences in membrane composition between the pathogen and the human host. Ergosterol, the predominant sterol of fungi, plants, and some protozoan parasites, interacts with Amphotericin B, resulting in an increased ion permeability of the membrane. Humans contain cholesterol, which has a low affinity for amphotericin B.

Miltefosine, an alkylphosphocholine derivative, is a new antileishmanial drug and the first effective oral treatment of visceral leishmaniasis. However, there are concerns regarding teratogenicity, rapid emergence of resistance, and variable cure rates, possibly due to species differences in drug sensitivity. The mechanism of action of miltefosine is not known.

The pentavalent antimonial drugs sodium stibogluconate and meglumine antimonate are prodrugs that require biological reduction to the trivalent form Sb(III) for antileishmanial activity. Sb(III) seems to inhibit the leishmanial trypanothione reductase, which, together with a depletion of thiols, results in a breakdown of the cellular thiol redox potential. *Leishmania* and other kinetoplastidae possess an unusual antioxidant termed trypanothione, which is composed of two molecules of glutathione joined by a spermidine linker. Trypanothione protects the cell from oxidative stress by reducing any disulfide bonds formed within proteins to cysteines. The trypanothione reductase regenerates oxidated trypanothione.

Clinical Use

Pentavalent antimonial drugs have been the cornerstone of antileishmanial therapy for more than 70 years, in spite of their general toxicity causing a wide range of side effects [2]. Pentavalent antimonial drugs have to be administered parenterally, which is a painful procedure. Meanwhile, resistance is widespread. In India,

pentavalent antimonial drugs have become almost obsolete because of resistance. They are still used in most other parts of the world where resistance has remained low. Alternative drugs are few and all have significant drawbacks:

1. *Amphotericin B*: This second line drug has moved into the first line in India.
2. *Liposomal Amphotericin B*: This is a highly effective drug against visceral leishmaniasis with remarkably few side effects. There is, however, only one producer and the price per treatment (US \$1,500) is beyond the reaches for most communities affected by the disease.
3. *Paromomycin*: This oral aminoglycoside was first shown to be effective as a topical treatment for cutaneous leishmaniasis, and later as a parenteral drug against visceral leishmaniasis. Phase III clinical trials were completed in 2005 in India, 15 years after the potential of this component for treating visceral leishmaniasis was discovered. It is currently not registered for this use.
4. *Miltefosin*: The antileishmanial activity of this anticancer drug was discovered in the mid-1980s. It is the first oral drug available to treat visceral and cutaneous/mucocutaneous leishmaniasis. However, the registration process is slow.

Antitoxoplasma Drugs

Infection with the obligatory intracellular parasite *T. gondii* is mainly acquired by ingestion of contaminated food or water. Approximately a third of the world's human population is infected.

Mechanism of Action

Clindamycin, a lincosamide derivative, inhibits protein biosynthesis within a unique organelle of the parasite, termed apicoplast. Its mode of action is similar to that of spiramycin.

The folate antagonists, pyrimethamine and sulfadiazine, inhibit the parasite's DHFR/TS synthase enzyme complex and the DHPS, respectively (Fig. 4) (see antimalarial drugs). To avoid deficiency of folic acid in patients treated with antifolate antagonists, folinic acid supplementation is recommended to reduce bone-marrow suppression.

Spiramycin is a macrolide that inhibits protein biosynthesis by blocking transfer of the aminoacyl-rRNA along the ribosome in a unique organelle of the parasite, termed apicoplast. The apicoplast is a remnant of a secondary endosymbiosis of a red algae and is only found in the phylum sporozoa.

Clinical Use

Toxoplasmosis remains a challenge to clinicians. *T. gondii* is one of the most prevalent parasites worldwide and it persists in the body for a lifetime.

The infection passes unnoticed, or with little signs and symptoms, in immunocompetent children and adults. The parasite poses a major threat, however, when acquired during pregnancy and transmitted to the fetus, and in immunocompromised patients with or without AIDS, due to reactivation of latent disease or newly acquired infection. The major clinical conditions are congenital toxoplasmosis, ocular toxoplasmosis, and toxoplasmic encephalitis. Current treatment options are confined to the acute stage, but do not eradicate the parasite from the patient. The most commonly used treatment, and currently probably the most effective, is the combination of pyrimethamine and sulfadiazine, supplemented by folinic acid to prevent bone marrow suppression [3]. In maternal infection during pregnancy, the primary goal is to prevent transmission to the fetus, the secondary goal, to treat the infected fetus at the earliest time possible to reduce damage. Due to the specific requirements during pregnancy, spiramycin is recommended for the first and early second trimester, and pyrimethamine/sulfadiazine for the late second and third trimester [3]. In most countries, treatment is continued in the newborn for various lengths of time. The efficacy of this regimen, however, has still to await confirmation by appropriately designed studies, and different drug regimens and strategies need to be tested for different clinical settings. In the immunocompromised patients, recrudescence after successful treatment of acute toxoplasmosis is a problem. If restitution of the immune response cannot be achieved or is, for therapeutic purposes, not desired, chemoprophylaxis needs to be installed.

Drugs Against African Sleeping Sickness

African sleeping sickness is a parasitic disease of increasing importance, with an estimated 300,000–500,000 cases annually. The etiological agents, *T. brucei gambiense* and *T. brucei rhodesiense*, are transmitted to humans by the bite of Tsetse flies.

Mechanism of Action

Eflornithine (difluoromethylornithine, DFMO) inhibits the ornithine decarboxylase of the polyamine pathway, in both the trypanosome and the mammalian cell, by acting as an irreversible competitor of the natural substrate ornithine. Inhibition of ornithine decarboxylase results in depletion of the polyamines, putrescine, spermidine and spermine, which are essential for cell proliferation. Eflornithine selectively harms the parasite and not the mammalian cells, despite acting as an ornithine decarboxylase inhibitor in both cell types. This selectivity is explained by the lower rate of ornithine decarboxylase production in the parasite, as compared to mammalian cells. Due to the high turnover rate, mammalian cells are capable of quickly replenishing inhibited ornithine decarboxylase by newly

synthesized enzyme. Eflornithine is only effective against *T. brucei gambiense*.

Melarsoprol, a trivalent organic melaminophenyl arsenic compound, kills intracerebral parasites of both *T. brucei gambiense* and *T. brucei rhodesiense*. Melarsoprol accumulates via an adenosine/adenine transporter in trypanosomes and is believed to inhibit glycolytic enzymes. Melarsoprol leads to a rapid lysis of trypanosomes. Melarsoprol is highly toxic to humans.

Pentamidine is an aromatic diamidine. Pentamidine uptake by the parasite is mediated by several different adenosine transporters. The mode of action is unclear.

Suramin, a symmetrical, polysulfonated naphthylamine, inhibits a number of trypanosomal enzymes; however, the importance of these effects on parasite killing is not clear.

Clinical Use

Despite great advances in the understanding of the biology of the parasite, progress in terms of drug development has been dreadfully slow.

1. Haemolympathic stage (acute stage): For the last two decades, the first line treatment for the acute stage are Pentamidine and Suramin [2]. One compound, the prodrug DB 289 (a diamidine), is currently in phase III clinical trials.
2. Central nervous system stage (late stage): Melarsoprol has remained the first line treatment for more than 20 years [2]. Melarsoprol is very toxic, and it is estimated that, of the patients treated for late-stage disease, an alarmingly high proportion suffers severe side effects or die. A cumbersome dosing schedule, used for decades, has recently been shortened to a 10-days course based on pharmacokinetic studies and controlled clinical trials. This certainly will improve patient compliance and costs. Eflornithine has joined as a second option to treat late-stage *T. gambiense* disease. Compared with melarsoprol, incidence and severity of adverse effects are much lower, however, application and manufacturing problems are the negative trade-offs. Currently, Nifurtimox, in combination with eflornithine, is in phase III clinical trials.

Drugs Against Chagas Disease

Chagas disease is a serious public health problem in Latin America, where an estimated 300,000 new cases occur each year. The causative agent is *T. cruzi*. *T. cruzi*, which is transmitted to humans by reduviid vectors. During acute disease, the parasite develops intracellularly in many tissues, including nervous and muscular tissue. Focal parasite-induced degeneration of infected organs, particularly the heart and the gastrointestinal tract, characterize the chronic form.

Mechanism of Action

Benznidazole, a nitroimidazole derivative, has a mode of action that seems to involve covalent modification of macromolecules by nitroreduction intermediates.

Nifurtimox, a nitrofuran, is a prodrug that is reduced to unstable nitroanion radicals, which react to produce highly toxic oxygen metabolites, such as superoxide and peroxide. Oxidative stress subsequently kills the parasite, which seems to lack effective enzymatic pathways to detoxify oxygen metabolites.

Clinical Use

Benznidazole and nifurtimox, which have been developed in the 1960s and 1970s, have remained the only two drugs available for the acute stage of the disease. There is no proven effect on the chronic stage, as recently reviewed by the Cochrane Collaboration. The specific treatment of the chronic stage has gained renewed interest with recent findings where persisting parasites may play a role in the development of irreversible lesions. The side effect of the two available compounds can be severe. Clinical trials have been carried out with allopurinol for the acute phase in the 1980s (phase II) and are currently in progress with benzimidazole for the indeterminate stage of the disease.

Drugs Against *E. Histolytica* and *G. Lamblia*

E. histolytica and *G. lamblia* are waterborne infectious diseases that cause colitis and liver abscess, and enteritis, respectively.

Mechanism of Action

5-Nitroimidazoles derivatives, such as metronidazole, tinidazole, ornidazole and secnidazole, are the drug of choice in the treatment of anaerobic protozoa. All 5-nitroimidazoles share the same mode of action. Anaerobic microorganisms reduce 5-nitroimidazoles to their active forms. This process only occurs under strongly reducing conditions. In some anaerobic protozoa and bacteria, such conditions are achieved when ferredoxin is reduced by the fermentation enzyme pyruvate ferredoxin oxidoreductase (POR). Ferredoxin can transfer one electron to 5-nitroimidazole, resulting in the reduction of the nitro group. POR does not occur in mammalian cells. The corresponding enzyme to POR in mammalian cells is pyruvate decarboxylase, which is not able to establish a reducing potential high enough for the reduction of 5-nitroimidazoles. The reduced products of 5-nitroimidazoles disrupt the DNA structure, thereby interfering with transcription and replication.

Clinical Use

E. histolytica colonization of the large intestine is eradicated using a luminal agent such as diloxanid furoate or paromomycin. Invasive amoebiasis (colitis,

liver abscess) is treated with one of the 5-nitroimidazole derivatives [4], followed by a luminal agent to prevent relapse from remaining cysts in the intestine.

G. lamblia is treated with 5-nitroimidazole derivatives. Paromomycin is a second choice in specific circumstances (e.g. pregnancy). In a Cochrane review, where 34 trials were included and where only one trial was without significant methodological flaws, the authors concluded that a single dose of tinidazole can provide the highest clinical cure rate with relatively few adverse effects. The high recurrence rate of disease after initial drug therapy is a problem.

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Antipsychotic Drugs

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Synonyms

Typical antipsychotic drugs: Neuroleptic drugs; conventional antipsychotic drugs; older antipsychotic drugs;

Atypical antipsychotic drugs: Novel antipsychotic drugs; serotonin/dopamine antagonists; 5HT_{2A}/D₂ antagonists.

Definition

Drugs that are effective in alleviating psychotic manifestations of a number of neurodegenerative and psychiatric disorders especially ►schizophrenia. Antipsychotic drugs are generally divided into two main groups based on their propensity to cause motor side effects and sustained elevation of plasma ►prolactin levels at clinically effective doses. Older or “typical” antipsychotic drugs (e.g. chlorpromazine and haloperidol) are associated with a high incidence of motor adverse effects and usually cause hyperprolactinemia. Newer antipsychotic drugs (clozapine, risperidone,

olanzapine, quetiapine, and ziprasidone) are called “atypical” antipsychotic drugs because they cause significantly lower motor side effects and usually avoid hyperprolactinemia.

Mechanism of Action

The serendipitous discovery of the antipsychotic effect of chlorpromazine in 1952 brought renewed hope in the treatment of psychotic disorders. The observation of chlorpromazine's antagonism of dopamine receptors heralded the introduction of multiple other antipsychotic drugs based on their common ability to cause catalepsy in laboratory animals leading to the dopamine hypothesis of schizophrenia. Central to the dopamine hypothesis is that all known antipsychotic medications, including atypical antipsychotic medications, bind to dopamine- D_2 receptors (D_2 -receptors). With the exception of aripiprazole, a partial agonist at D_2 -receptors, ▶[positron emission tomography](#) (PET) studies have shown a direct relationship between central D_2 -▶[receptor occupancy](#) and clinical effects of antipsychotic medications, with clinical response occurring only when at least 60% of central D_2 -receptors are occupied while ▶[extrapyramidal side effects](#) (EPS) occur at D_2 -receptor occupancy above 80% [1]. Antipsychotic doses resulting in D_2 -receptor occupancy higher than 80% result in more adverse effects with no additional clinical benefit, consistent with earlier observations that increasing the antipsychotic medication beyond this ‘neuroleptic threshold’ resulted in no additional benefit other than possibly decreasing clinical measures of hostility.

The introduction of clozapine presented a major challenge to the dopamine hypothesis. Not only does it show a modest clinical superiority over older antipsychotic agents (i.e. it is effective in some patients who do not respond to older antipsychotic drugs), but it does so without causing EPS or hyperprolactinemia. Pharmacologically, clozapine has been shown to have a low affinity for dopamine D_2 -receptors (resulting in 20–70% occupancy) and a high affinity for serotonin ($5HT_2$) receptors (>80% occupancy). This gave rise to the hypothesis that its atypical nature was related to its high $5HT_2$ -receptor (specifically $5HT_{2A}$ -receptor) affinity relative to its low D_2 -receptor affinity [2]. The role of $5HT_{2A}$ antagonism in the uniqueness of clozapine has been challenged [1]. A number of trials using drugs known to be antagonists of the $5HT_{2A}$ -receptor (but not of dopamine receptors) have failed to show clozapine-like efficacy. Similarly, the $5HT_{2A}$ -receptors are saturated by clozapine at sub-therapeutic doses, indicating that $5HT_{2A}$ -receptor antagonism is not sufficient to effect an antipsychotic response. There are some case reports suggesting that augmentation of older antipsychotic medications with specific $5HT_2$ -receptor antagonists may potentiate the antipsychotic efficacy, but this remains to be tested in controlled clinical trials.

Recently there has been interest in modulating release of prefrontal dopamine (putative improved cognitive function) using antagonists or inverse agonists at other serotonin receptors including $5HT_{2C}$ and $5HT_6$ receptors, though these studies remain at the preclinical stage. In summary, it has been suggested that clozapine's low EPS and avoidance of hyperprolactinemia is attributable to $5HT_2$ antagonism, but some degree of D_2 -receptor antagonism may still be necessary for an antipsychotic response.

The $5HT_2/D_2$ hypothesis was influential in the introduction of four new antipsychotic medications (risperidone, olanzapine, quetiapine, and ziprasidone), all having in common a high ratio of $5HT_2/D_2$ receptor affinity and lower incidence of both EPS and hyperprolactinemia [2]. Paliperidone, a recently introduced antipsychotic, is the active metabolite of risperidone (5-hydroxyrisperidone), with a similar binding profile to the parent drug. At least in the case of risperidone and olanzapine (and possibly also ziprasidone), this ‘atypical’ nature appears to be lost in a dose-dependant manner resulting in the appearance of EPS and sustained hyperprolactinemia at higher doses. Indeed, the relationship between dopamine D_2 -receptor occupancy and clinical effects (response and EPS) for risperidone and olanzapine in human subjects studied with PET is very similar to that found with older antipsychotic drugs (i.e. a threshold >60% D_2 -receptor occupancy for clinical response and >80% D_2 -receptor occupancy for EPS). On the other hand, clozapine and quetiapine are clinically effective at lower D_2 -receptor occupancy without showing sustained hyperprolactinemia. Some studies evaluating the ratio of D_2 -receptor occupancy in extrastriatal regions relative to the striatum suggested that atypical antipsychotic medications preferentially bind to extrastriatal (i.e. limbic) D_2 -receptors when compared with typical antipsychotic drugs. However, data from a study using kinetic analysis (a technique that avoids a number of the limitations of ratio studies) in non-human primates is not consistent with this hypothesis. The dual observation that antagonism of dopamine D_2 receptors is associated with both clinical antipsychotic effects as well as adverse motor (EPS) and subjective (anhedonia) effects, partial agonists at D_2 receptors have been a focus of drug development, leading to the first drug in this category on the market (aripiprazole). The intrinsic efficacy of the partial agonist at the D_2 receptor is thought to be critical to produce adequate antagonism without deleterious stimulation of the D_2 receptors leading to worsening of psychotic symptoms. The experience with aripiprazole, with an intrinsic efficacy of circa 30%, has shown this drug to be an effective antipsychotic with minimal EPS notwithstanding very high central D_2 occupancy in excess of 90%, consistent with its partial agonist effects.

Recent studies suggest that the apparent low striatal D₂-receptor occupancy may be a result of quetiapine and clozapine's loose binding to D₂-receptors (i.e. high k_{off} resulting in low ▶affinity for D₂-receptor) [1]. Hence endogenous dopamine and low concentrations of radioligands (used in these experiments) may displace an appreciable amount of bound drug resulting in underestimation of D₂-receptor occupancy. In one study, patients treated with quetiapine at doses ranging from 300 to 600 mg/day showed normal prolactin levels and less than 20% D₂-receptor occupancy 12 h after their last dose. However, transiently elevated prolactin levels and appreciable (64%) dopamine D₂-receptor binding were noted 2 h after drug administration. Similarly clozapine (350 mg/day) resulted in 71% D₂-receptor occupancy 1–2 h after administration, declining to 55% and 26% after 12 and 24 h, respectively. While these findings await replication, they raise the possibility that different pharmacodynamic properties of dopamine receptor antagonists may be sufficient to explain their varying degrees of 'atypicality' [3]. The transient dopamine receptor occupancy may also account for clozapine's clinical superiority, since it has been shown that repeated transient dopamine receptor antagonism results in sensitization of the dopamine system, while continuous receptor antagonism results in tolerance and up-regulation of the system.

While all antipsychotic medications have a robust acute effect on delusions, auditory hallucinations, and disorganized behaviour (also known as 'positive symptoms'), and maintenance treatment has been shown to decrease both relapse and hospitalization rates, their effect on negative symptoms (apathy, avolition, alogia, and affective flattening) and related cognitive disturbance (e.g. attentional problems and disrupted working memory) is at best marginal. Newer antipsychotic medications, especially clozapine, have been shown to have some effect on negative symptoms and selected cognitive measures when compared with older antipsychotics such as haloperidol, but this topic remains controversial due to the difficulty in distinguishing primary negative symptoms from secondary (i.e. adverse) effects of the (older) medications [3]. In vivo measurements of extracellular dopamine levels using microdialysis in rodents and primates have shown that while both acute administration of clozapine and haloperidol result in an increase in dopamine levels in the striatum, clozapine results in higher dopamine levels in the prefrontal cortex compared with haloperidol. With chronic administration of clozapine in rodents, the increased dopamine release is maintained only in the prefrontal cortex but not in the striatum. It has been postulated that this modulation of prefrontal dopaminergic transmission may be involved in its effects on cognitive and negative symptoms, which are known to be associated with decreased prefrontal

activity in functional neuroimaging studies [4]. Current approaches to drug development are focused on modulation of prefrontal dopaminergic activity directly (e.g. D1 agonism), indirectly via dopaminergic mechanisms (e.g. dopamine reuptake inhibition) or serotonergic mechanisms (e.g. 5HT_{2C} and 5HT₆ antagonism) – this focus is part of a growing realization that the 'antipsychotic' effect of currently available antipsychotics is largely limited to positive symptoms, while negative and cognitive symptoms are associated with functional outcome, remain unresponsive to D₂ antagonists. Other approaches to cognitive enhancement also involves modulation of muscarinic M1 receptors (e.g. desmethylclozapine, a metabolite of clozapine with M1 agonist properties), nicotinic receptors (in particular α -7-nAChR), NMDA receptors (e.g. glycine and other NMDA receptor modulators), and metabotropic glutamate receptors (e.g. mGluR5 and mGluR2). These mechanisms are unlikely to be associated with effects on positive symptoms, though some downstream effects may also be related to prefrontal dopamine release (e.g. M1 agonists and medial prefrontal cortical release of dopamine) so that the effects may well be convergent mechanistically notwithstanding the targeting of different receptors.

It is thought that the mesolimbic dopaminergic projections from ventral tegmental area (VTA) are involved in the clinical response to antipsychotic drugs, and that in contrast to older antipsychotic drugs, newer antipsychotic drugs may act preferentially on these neurons. Acute administration of haloperidol in anaesthetized rodents has been shown to increase firing rate of neurons in the substantia nigra (SN) as well as the VTA. On the other hand daily administration for 3 weeks leads to a decline in the activity in these dopaminergic neurons below that at baseline, an electrophysiological phenomenon known as 'depolarization block'. All antipsychotic medications have the ability to cause depolarization block in the VTA, and their ability to cause depolarization block in the SN is related to their propensity to cause EPS in human subjects. Clozapine causes depolarization block in the VTA but not in the SN, consistent with involvement of the mesolimbic system in its antipsychotic effect [5].

In summary, the mechanism of action of antipsychotic drugs appears to be intricately linked with the normalization of a disrupted state of dopaminergic transmission. Remission of positive symptoms and the emergence of extrapyramidal side effects are associated with specific levels of striatal dopamine D₂-receptor occupancy. An increased ratio of 5HT₂/D₂-receptor antagonism and/or altered pharmacodynamic properties of atypical antipsychotic drugs resulting in loose binding to D₂-receptors may be involved in the decreased incidence of motor side effects with some newer antipsychotic drugs, while antipsychotic action may involve activity of these drugs on the mesolimbic

ascending dopaminergic neurons. Preferential activity and modulation of prefrontal dopaminergic activity by atypical antipsychotic drugs may be related to their effects on cognitive and negative symptoms of schizophrenia.

Clinical Use (Including Side Effects)

Antipsychotic medications are indicated in the treatment of acute and chronic psychotic disorders. These include schizophrenia, schizoaffective disorder, and manic states occurring as part of a bipolar disorder or schizoaffective disorder. The co-administration of antipsychotic medication with antidepressants has also been shown to increase the remission rate of severe depressive episodes that are accompanied by psychotic symptoms. Antipsychotic medications are frequently used in the management of agitation associated with delirium, dementia, and toxic effects of both prescribed medications (e.g. L-dopa used in Parkinson's disease) and illicit drugs (e.g. cocaine, amphetamines, and PCP). They are also indicated in the management of tics that result from Gilles de la Tourette's syndrome, and widely used to control the motor and behavioural manifestations of Huntington's disease.

The choice of antipsychotic medications is largely dependent on considerations related to their individual side effect profile. Older antipsychotic medications are generally divided into high, moderate, and low potency drugs, potency being related to their propensity for causing EPS. ► **Tardive dyskinesia** is the most common and potentially most disabling long-term side effect. The neuroleptic malignant syndrome is the most severe neurological side effect and consists of hyperthermia, autonomic instability, and muscle stiffness that may result in dehydration, renal failure, and death. Early diagnosis and management has resulted in decreased mortality from this condition. In addition to neurological side effects, other systems may also be affected by typical antipsychotic medications. In contrast to the neurological side effects, the incidence of these side effects are generally inversely proportional to the potency of the drug used. These include autonomic effects (e.g. tachycardia, dry mouth, urinary retention, constipation), haematologic effects (e.g. neutropenia and rarely agranulocytosis), neurological (sedation, seizures), endocrine (e.g. weight gain, galactorrhoea, obesity, hypercholesterolemia, and hyperglycemia), and dermatological effects (e.g. photosensitivity).

The atypical antipsychotic drugs were introduced with the goal of minimizing neurological adverse effects associated with older antipsychotic medications. However, these medications are not free of serious adverse effects including dose-related parkinsonism (risperidone and olanzapine), dose-related risk of seizures (clozapine), endocrinological manifestations (including diabetes, weight gain, and hypercholesterolemia), and

haematological abnormalities (neutropenia and agranulocytosis with clozapine). Nonetheless, while older antipsychotic medications remain the most widely used antipsychotic medications globally, the use of newer antipsychotic medications has largely dominated the market in Western Europe and North America. The principle clinical advantage that has led to this shift in prescribing practice is undoubtedly the decreased incidence of neurological side effects, which are associated with significant morbidity and poor outcome largely secondary to non-compliance. Indeed, they are generally recommended as first line agents in the treatment of psychotic disorders, with typical antipsychotic drugs reserved for patients having previously been successfully maintained on these medications or requiring parenteral antipsychotic drugs (e.g. short-acting intramuscular neuroleptics for agitation, and long-acting 'depot neuroleptics' for patients who are non-compliant with oral medication). While atypical antipsychotic medications are more acceptable to patients, their impact on the long-term outcome of schizophrenia remains to be established. Moreover, continued vigilance for their potentially significant long-term side effects including obesity, hypercholesterolemia, and impaired glucose tolerance is warranted. While all antipsychotic drugs may be associated with obesity and metabolic syndrome, generally the likelihood for these effects are highest for clozapine and lowest for ziprasidone and aripiprazole in the following order:

clozapine > *olanzapine* > *risperidone*, > *quetiapine*
> *ziprasidone* and *aripiprazole*.

All antipsychotic medications are effective in alleviating positive and negative symptoms of schizophrenia, while atypical antipsychotic agents have been associated with some superior efficacy in reduction of negative symptoms. Following an adequate trial of antipsychotic treatment (8–12 weeks of treatment with adequately dosed antipsychotic drug) in acute schizophrenia, 60% of patients show significant improvement or remission compared with 20% of patients treated with placebo. Of the 40% who do not respond, approximately half respond to subsequent trials with other antipsychotic medications. The acute phase of the illness is treated with an oral antipsychotic medication titrated to the appropriate clinically effective dose. This may be supplemented by short-acting intramuscular antipsychotic agents in patients in whom rapid sedation is clinically indicated (e.g. severe agitation). Patients failing to respond to an adequate trial of an antipsychotic medication should be switched to another antipsychotic drug from a different pharmacological class. Patients who fail to respond or show only partial response to two adequate trials of antipsychotic drugs (including at least one atypical antipsychotic drug) or experience severe neurological side

effects (e.g. tardive dyskinesia) should be considered for a trial of clozapine [6]. Significant inter-individual variability in dose requirements is commonly seen, and this may be influenced by sex, age, and concomitant medications. Increasing the dose beyond which causes extrapyramidal side effects results in no additional clinical advantage. The augmentation with a second antipsychotic (antipsychotic polypharmacy) has not been supported by randomized controlled trials, and while this is very common in clinical practice, the practice should be reserved after other therapeutic strategies have failed and certainly not before a trial of clozapine. Regular monitoring for weight gain, hypercholesterolemia, triglycerides, and fasting blood sugars, as well as extrapyramidal side effects is warranted especially during the first 3 months of treatment, and then every 6 months or following any medication or dose changes. Patients are maintained on the clinically effective dose for the next 3–6 months. Following the resolution of the acute phase, the dose may be decreased to address any adverse effects having significant functional or emotional impact on the patient's well-being.

The duration of treatment is generally considered to be indefinite in patients diagnosed with schizophrenia, though selected patients recovering from a first psychotic episode may be considered for gradual taper of medication after at least 1-year of treatment and with close psychiatric follow up. Patients with a history of multiple psychotic episodes should be stable for at least 5 years before considering a trial off medication. When considering discontinuation of antipsychotic treatment, an individualized approach is recommended with careful consideration of certain aspects of the disease course (e.g. severe occupational impairment when acutely ill, history of suicidal attempts and violence). Dose discontinuation is associated with a very high 2-year relapse rate up to 60% in chronically treated patients, though most relapses occur within the first 3 months of discontinuation. If drug discontinuation is to be attempted, gradual taper and very close monitoring is critical, and in some studies has not been associated with longer duration of hospitalization. Symptom-targeting strategies and drug holidays have been tried in the past, but for most patients this is no longer recommended since it is associated with very high relapse rates. Finally, with the resolution of the acute phase of the psychotic episode, psychosocial, occupational, and cognitive difficulties need to be addressed, since they usually persist with significant impact on the patients' functional status.

In summary, antipsychotic drugs have a significant impact on the acute resolution and the maintenance of remission of symptoms of schizophrenia, enabling focus on rehabilitation efforts directed at residual cognitive, social, and occupational disabilities. The

advent of atypical antipsychotic drugs brought lesser motor side effects and renewed hope to patients and families affected by this devastating illness, though these newer agents incur a variable degree of risk of weight gain and metabolic syndrome which needs to be carefully monitored. It is hoped that a better understanding of pharmacological mechanisms underlying the clinical superiority of drugs like clozapine will lead to the development of new treatment strategies with better efficacy and improved side effect profile.

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Antipyretic Agents

Antipyretic agents are used for the treatment of fever. The most commonly used antipyretics are acetylsalicylic acid and paracetamol (synonym acetaminophen).

► Non-steroidal Anti-inflammatory Drugs

Antiretroviral Agents

► Antiviral Drugs

Antirheumatoid Drugs

Antirheumatoid drugs are employed in the treatment of rheumatoid disease (rheumatoid arthritis). The

characteristic feature of this autoimmune disease is a persistent inflammation of peripheral joints. The inflammatory process leads to joint damage and subsequently to marked functional impairment. Inflammatory cytokines play a major role in the pathogenesis of the disease. Drugs used in the therapy of rheumatoid arthritis are non-steroidal anti-inflammatory drugs, glucocorticoids, immunosuppressive agents and disease-modifying antirheumatoid drugs (DMARDs). DMARDs are not analgesic, but they suppress the inflammatory process. DMARDs include drugs with cytotoxic and immunosuppressant activity (azathioprine, cyclosporin, methotrexate), gold compounds (auranofin and sodium aurothiomalate), anti-malarial drugs (chloroquine and hydroxychloroquine) and sulphasalazine. The last is also used for the treatment of chronic inflammatory bowel disease.

- NSAID
- Glucocorticoids
- Immunosuppressive Agents
- Inflammation

Antisense DNA

- Antisense Oligonucleotides (ASON)

Antisense Oligonucleotides

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Synonyms

Antisense DNA; Reverse complementary oligonucleotides; ASON

Definition

Antisense therapy means the selective, sequence-specific inhibition of gene expression by single-stranded DNA oligonucleotides. By hybridizing to the target mRNA, which results in a subsequent double-helix formation, gene expression is blocked. This process can occur at any point between the conclusion of transcription and initiation of translation or even possibly during translation.

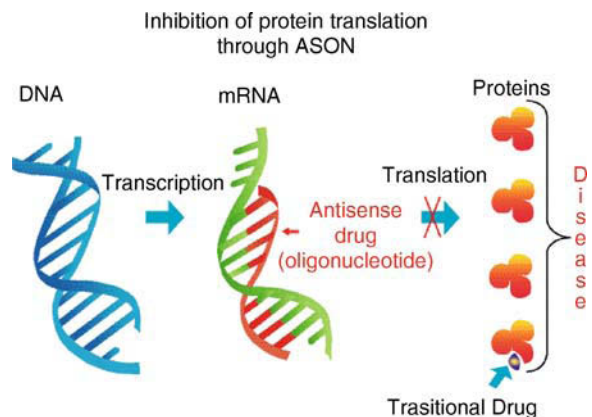
Description

ASON are sequences of usually 17–30 bases of single-stranded DNA that hybridize to specific genes or their mRNA products by Watson–Crick base pairing and disrupt their function. In the case of AS-ODN (antisense oligodeoxyribonucleotides) cellular RNaseH is able to bind to the DNA–RNA duplex and hydrolyze the RNA, resulting in increased transcript turnover. Modifications to the deoxy moiety at the 2'-sugar position prohibits RNase H action.

In rarer cases the ODNs also prevent normal gene transcription by directly forming triplex-helix structures with target DNA. This does not destroy a gene but prevents its unwinding or its binding to a gene promoter.

The short length of a typical ASON facilitates cell internalization and increases hybridization efficiency by reducing base-mismatch errors. Once hybridization has occurred the ASON–mRNA complex becomes a substrate for intracellular ►RNases (e.g., RNase-H) that catalyze mRNA degradation and allow ASON to recycle for another base pairing with the next target mRNA molecule. The net result of this process is a sustained decrease of target mRNA translation and a lower intracellular level of the corresponding protein (Fig. 1).

The therapeutic utility of systemically administered ASON had been limited by their short plasma half life (sometimes even less than 3 min). This is due to their sensitivity to nuclease digestion. When the first-generation ASON were chemically modified, e.g., by replacing the oxygen in the phosphodiester bond with sulfur (phosphorothiorate) they obtained an increased stability in biological fluids while their antisense effect has been maintained. First-generation agents can be delivered via intravitreal injection, parenterally, by topical cream, enema, and inhaled aerosol. These antisense



Antisense Oligonucleotides. Figure 1 Schematic representation of the action of antisense oligonucleotides. They bind to their respective target mRNA preventing protein translation.

drugs have a sulfur chemistry modification that makes the drug more resistant to degradation, increasing stability in the blood stream and in tissues. This specific chemical modification prevents the rapid elimination of the drug from the body.

The second-generation chemistry adds 2'MOE modifications to the sulfur modification of first-generation chemistry. In turn, second-generation drugs have increased target-binding affinity and resistance to degradation. Second-generation drugs are composed of both RNA-like and DNA-like nucleotides, while first-generation drugs are entirely DNA-like. Because RNA hybridizes more tightly to RNA than to DNA, the second-generation drugs have a greater affinity for their RNA targets and, therefore, greater potency.

Another new modification is the 2'-deoxy-2'-fluorodarabinonucleic acid (2'F-ANA), which increases the strength of the oligonucleotide-mRNA hybrids, elicits efficient RNase H-mediated degradation of the target, is more nuclease resistant and reaches high intracellular concentrations for prolonged time. Similar results could be obtained with oxetane modified ASONs.

A problem with employment of ASON in a larger clinical setting is their poor uptake and inappropriate intracellular compartmentalization, e.g., sequestration in endosomal or lysosomal complexes. In addition, there is a need for a very careful selection of the ASON-mRNA pair sequences that would most efficiently hybridize. To date, several computer programs are used to predict the secondary and tertiary structures of the target mRNA and, in turn, which of the mRNA sequences are most accessible to the ASON. However, even with this sophisticated techniques, the choice of base-pairing partners still usually includes a component of empiricism. Despite these principal limitations, it has become clear that ASON can penetrate into cells and mediate their specific inhibitory effect of the protein synthesis in various circumstances.

The basic concept of the use of ASON can be modified in several ways:

1. Antisense RNA that is expressed intracellularly following transfection with antisense genes.
2. ► **Ribozymes** that are small RNA molecules with endoribonuclease activity and exhibit catalytic sequence-specific cleavage of the target.
3. The ribozymes were widely modified and can be further subdivided according to their structural features in group I ribozymes, hammerhead ribozymes, hairpin ribozymes, ribonuclease P (RNase P), and hepatitis delta virus ribozymes.

Pharmacological Relevance

Examples of Applied ASON Therapeutics

The number of clinical trials ongoing represents a growing interest in antisense technology.

1. ASON to inhibit angioplasty restenosis. Patients suffering from coronary stenosis can successfully be treated by percutaneous transluminal coronary angioplasty (PTCA). However, in up to 50% of the patients restenoses occurs necessitating a repeated PTCA: ASON emerged as a potentially useful strategy to prevent such restenoses in animal models and fist clinical trials are currently under progress [1].
2. ASON against HIV infection. Once HIV has infected the cell, the genomic RNA of the retrovirus is used to code for a double-stranded cDNA intermediate. This cDNA is integrated into the genome of the host cell by the viral integrase. RNA identical to the genomic RNA of the virus will be transcribed from the DNA of this provirus by the infected cell. In experimental systems, ASON were used to target various parts of the viral life cycle, e.g., genomic RNA reverse transcription, viral mRNA transcription, and viral translation. With this regard, GEM 91, a 25mer ASON against the HIV-1 gag gene has extensively studied. Newer oligonucleotide analogs are now available, which act as strong steric block agents of HIV RNA function. In ongoing studies targeting the HIV-1 *trans*-activation responsive region (TAR) and the viral packaging signal (ψ) with steric block oligonucleotides of varying chemistry demonstrate their great potential for steric blocking of viral protein interactions in vitro and in cells and describe the first antiviral studies [2].
3. ASON for targeting the Bcl-2 proto-oncogene in human cancers. The Bcl-2 protein is a major-apoptosis inhibitor originally identified by its involvement of a chromosomal translocation t(14;18) found in follicular Non-Hodgkin Lymphoma. Beside lymphomas, bcl-2 is up-regulated in several other tumors, e.g., leukemia, breast cancer, melanoma, prostate cancer, small and non-small lung carcinoma. In most of these studies, an 18-mer phosphothiorate ASON targeting the first six codons of bcl-2 (ISIS G3139) was used. The bcl-2 antisense therapy was feasible and showed potential antitumor activity. However, the mean inhibition of bcl-2 expression was only moderate and the clinical significance of this small decline was uncertain. Besides bcl-2, a large variety of other ► **oncogenes** have been targeted in cancer cell models. Table 1 gives an overview of such attempts. An emerging understanding of the most effective treatment setting – to test the addition of oblimersen to other therapies – has come from recent reports of two randomized studies in melanoma and CLL. In the melanoma study, the addition of oblimersen to dacarbazine produced a survival benefit for patients with normal LDH. In the CLL study, the addition of oblimersen to fludarabine and cyclophosphamide significantly increased durable CR and nPRs,

Antisense Oligonucleotides. Table 1 Malignant disorders as potential targets for ribozyme gene therapy

Target gene	Gene product	Ribozyme-induced change of function
<i>bcr-abl</i>	Tyrosine kinase	Inhibition of cell proliferation and colony formation
<i>PML/RAR α</i>	Transcriptional regulator	Inhibition of cell proliferation; induction of apoptosis; increase in sensitivity against ATRA
AML1/MTG8	Transcription factor	Inhibition of cell proliferation; induction of apoptosis
N-ras, H-ras, K-ras	Signal transduction pathway	Inhibition of cell proliferation and colony formation; change in morphology, enhanced melanin synthesis; decrease of in vivo tumorigenicity
EGFR	Receptor tyrosine kinase	Inhibition of cell proliferation and colony formation; decrease of in vivo tumorigenicity
<i>c-erbB-2</i> (HER2/ <i>neu</i>)	Receptor tyrosine kinase	Inhibition of cell proliferation; decrease of in vivo tumorigenicity
<i>c-erbB-4</i>	Receptor tyrosine kinase	Inhibition of mitogenesis and colony formation; decrease of in vivo tumorigenicity
Estrogen receptor	Transcriptional regulator	Inhibition of cell-cycle progression
Androgen receptor	Transcriptional regulator	Inhibition of androgen receptor transcriptional activity
<i>c-fms</i>	Growth factor receptor	Inhibition of cell proliferation
RET	Receptor tyrosine kinase	Inhibition of colony formation
<i>Mdr-1</i>	Drug-efflux pump	Reduction in resistance to chemotherapeutic drugs
<i>c-fos</i>	Transcriptional regulator	Change in morphology; reduction in resistance to chemotherapeutic drugs
CD44	Cell adhesion molecule	n.d.
VLA-6	Adhesion receptor	Decrease of in vitro invasion and in vivo metastatic ability
MMP-9	Matric metalloproteinase	Decrease of in vivo metastatic ability
<i>CAPL</i> (S100A4)	Calcium-binding protein	Decrease of in vitro invasion; reduction in expression of MMP-2, MT1-MMP, and TIMP-1; decrease of in vivo metastatic ability
Pleiotrophin	Growth factor	Inhibition of colony formation; decrease of in vivo tumor growth, tumor angiogenesis, and metastatic ability
VEGF-R1/VEGF-R2	Growth factor receptors	Decrease of in vivo tumor growth; decrease of in vivo metastatic ability (VEGF-R2 only)
VEGF	Growth factor	n.d.
bFGF-BP	bFGF-binding protein	Reduction of release of biologically active bFGF; decrease of in vivo tumor growth and tumor angiogenesis
Telomerase	Ribonucleoprotein	Suppression of telomerase activity; inhibition of cell proliferation; change in morphology; induction of apoptosis
<i>Bcl-2</i>	Anti-apoptotic protein	Induction of apoptosis
PKC-α	Anti-apoptotic protein	Induction of apoptosis

with the greatest benefit occurring in patients with chemosensitive disease. Based on these considerations, a future randomized study of GO and oblimersen would be most likely to demonstrate the benefit of oblimersen in the setting of patients who had not previously been exposed to chemotherapy [2–4].

- Formivirsen to treat cytomegalovirus-induced retinitis in HIV-infected patients. The first antisense drug approved by the US Food and Drugs Administration (FDA) was formivirsen (ISIS 2922) that targets the CMVIE2 protein. Formivirsen was approved for the treatment of cytomegalovirus-induced retinitis in patients with AIDS. One or both eyes can be affected

Antisense Oligonucleotides. Table 2 Viral disorders with malignant complications as potential targets for ribozyme gene therapies

Virus	Malignant complication	Target gene for ribozymes	Ribozyme-induced change of function
Human papilloma virus (HPV)	Cervical cancer, oral cancer	E6, E7	Inhibition of cell proliferation and colony formation
Epstein–Barr virus (EBV)	Burkitt's lymphoma, nasopharyngeal carcinoma, lymphoproliferative disorders in immuno-suppressed patients (e.g., AIDS, transplant recipients)	EBNA-1	Inhibition of cell proliferation
Hepatitis B virus (HBV)	Hepatocellular carcinoma	Progenomic RNA	Inhibition of viral gene expression
Hepatitis C virus (HCV)	Hepatocellular carcinoma	5'-untranslated/cor region	Inhibition of viral gene expression

and it is not unusual for patients to suffer from severe visual impairment of even blindness as a result of untreated infections. However, the conventional treatment of CMV-retinitis also remains problematic, in particular for patients who cannot take, do not respond or become resistant to standard therapy by ganciclovir, foscarnet, and cidofovir. The main drawback of formivirsen is its need for local administration by intravitreal injection. Of note, the inhibitory effect of formivirsen for cytomegalovirus replication *in vitro* is about 30 times higher than for ganciclovir, the conventional treatment of choice for CMV infection.

- For ISIS 301012, a second-generation antisense inhibitor of apoB-100 in November 2006. Isis announced results from two Phase 2 clinical trials of ISIS 301012. In the first study reported, patients with high cholesterol on stable doses of statins were treated with ISIS 301012 for 5 weeks. Patients who received 300 mg/week of ISIS 301012 in this study achieved a 51% reduction in LDL-cholesterol (LDL), a 42% reduction in total cholesterol (TC), and a 41% reduction in triglycerides (TG) beyond the levels achieved with statins alone.
- ASON are able to regulate the sonic hedgehog pathway by down-regulating Gli2 (glioma associated oncogene), which plays a predominant role in the proliferation of HCC (hepatocellular carcinoma) cell lines. The suppression of Gli2 expression may provide a useful therapeutic option for the treatment of HCC [5].

Table 2 summarizes current ASON-mediated therapies against viral infections.

Non-Sequence Specific Activities of ASON

A rather unexpected stimulation of lymphocyte proliferation by ASON was frequently observed. Of note, the phosphorothiorate backbone of a given ASON

has immune stimulatory properties itself, which are independent of its DNA sequence. In contrast, the stimulatory effects of unmodified oligonucleotides are dependent of a simple unmethylated **▶CpG dinucleotide motif**. The increasing number of **▶CpG motifs** generally increases the level of activation of B-lymphocytes. In addition, ASON may have effects of cytokine or immunoglobulin secretion or may alter the DNA binding activity of transcription factors. These nonantisense immune-enhancing (or sometimes immune-suppressing) effects are generally recognized as an undesirable side-effect. However, they may have therapeutic utility by their own, even though the mechanisms are not yet fully understood.

In general, systemic treatment with ASON is well-tolerated and side effects are dose-dependent. Among those, thrombocytopenia, hypotension, fever, increasing liver enzymes, and complement activation were most frequently seen.

▶Cholinesterases

▶RNA Interference (RNAi)–SiRNA

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Antithyroid Drugs

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Synonyms

Thionamides

Definition

Antithyroid drugs block thyroid hormone production in the thyroid gland. Based on their mechanism of action antithyroid drugs are defined as: (i) thionamides, which block thyroid hormone production by inhibition of thyroid peroxidase mediated iodination of tyrosine residues of thyroglobuline. (ii) aniones, which inhibit the transfer of iodine into the thyroid gland, e.g. perchlorate. (iii) high-dose (g range) iodine as lugol's solution or as oral radiographic contrast agents that block the release of thyroid hormones for days with subsequent escape. Other drugs utilized to treat hyperthyroidism are β -blocking agents, and seldom lithium.

Thionamides are heterocyclic compounds that contain a thiourylene group. Thiouracil was the first widely used antithyroid drug. Further studies led to the introduction of substances with fewer side effects. Three drugs of this type are currently in use: methimazole, carbimazole and propylthiouracil [1]. Thiouracil and propylthiouracil belong to the subgroup of pyrimidines, whereas methimazole and carbimazole belong to the thioglyoxalines. The goitrogenic properties of several substances were first recognized in the 1940s when thyroid enlargement was noticed in rats that had been given sulfaguanine to study the antibiotic effects of sulfaguanidine on the intestinal flora [1]. Others noted the development of goiters in rats fed with phenylthiocarbamide. Later it was concluded that the effect of thiourea and the sulfonamides was due to the inhibition of thyroid hormone synthesis. It was first suggested that the entire thiourylene grouping (NH.CS.NH) would be necessary for antithyroid activity. Further studies revealed that only the thiocarbamide group (S = C–N) is essential for antithyroid activity.

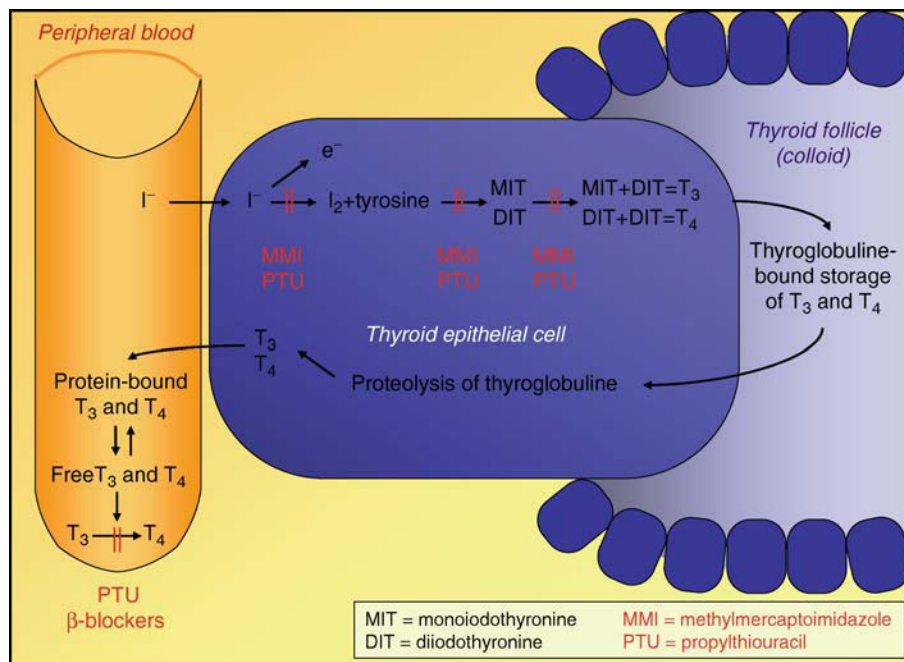
Mechanism of Action

Synthesis of thyroid hormones occurs in several steps. At first, inorganic iodide is actively concentrated by

thyroid follicular cells by the sodium iodide symporter. After oxidation to iodine (= iodination) it is bound to tyrosine residues thus forming monoiodothyronine (MIT) or diiodothyronine (DIT). MIT and DIT are coupled to form **triiodothyronine** (T₃) or **thyroxine** (T₄). Both iodination and coupling occur at the apical membrane of the thyroid follicular cell and within the **thyroglobuline** (Tg) molecule, and are catalyzed by the enzyme **thyroid peroxidase** (TPO). TPO is a haemoprotein enzyme with binding sites for both iodine and tyrosine. In model systems, TPO has no catalytic activity in the absence of H₂O₂. Therefore, it is assumed that H₂O₂ production is important also for thyroid hormone formation in vivo. TPO degrades H₂O₂ in a catalase-like reaction releasing O₂. Several iodination intermediates were postulated for this reaction, for instance TPO-bound iodinium (I⁺) and TPO-bound hypoiodite (I⁻). T₃ and T₄ are stored in the follicular lumen bound to thyroglobuline. The re-entry of Tg into the thyroid follicular cell involves a macropinocytosis process. Thyroid hormones are released after proteolysis of Tg. Type I and type II 5' deiodinase generate the active hormone T₃ by reductive deiodination of the phenolic ring of T₄ [2] (Fig. 1).

Antithyroid drugs inhibit the thyroid peroxidase-mediated iodination and coupling. Inactivation of TPO by antithyroid drugs involves a reaction between the drugs and the oxidized heme group produced by the interaction between TPO and H₂O₂. Results of several studies suggested that antithyroid drugs bind to the enzyme either at the same site as iodide or at a nearby site, and that the binding interferes with the binding of iodide [1, 2]. The type of inhibition depends on the extent of TPO inactivation and drug oxidation. These rates depend mainly on the iodine to drug concentration ratio. At a high iodine to drug ratio the inhibition of iodination is reversible and TPO is only partially inactivated. Under these conditions extensive drug oxidation occurs. When the iodine to drug ratio is low, iodination is irreversibly inhibited. This is associated with rapid and complete inactivation of TPO [2].

Propylthiouracil (PTU), but not methyl-mercaptoimidazole (MMI), has an additional peripheral effect. It inhibits the monodeiodination of thyroxine to triiodothyronine by blocking the enzyme 5' monodeiodinase [1]. In humans the potency of MMI is at least 10 times higher than that of PTU, whereas in rats PTU is more potent than MMI. The higher potency of MMI in humans is probably due to differences in uptake into the thyroid gland and subsequent metabolism, because in vitro inhibition of thyroid peroxidase by MMI is not significantly more potent than by PTU [1, 6]. Whether antithyroid drugs have additional immunosuppressive actions is a matter of discussion [1, 2].



Antithyroid Drugs. Figure 1 Synthesis and secretion of thyroid hormones and mechanisms of action of antithyroid drugs. Iodine is actively concentrated by the thyroid gland (sodium iodide transporter). After oxidation it is bound to tyrosine residues thus forming monoiodothyronine (MIT) or diiodothyronine (DIT). MIT and DIT are coupled to triiodothyronine (T₃) or thyroxine (T₄) and are stored in thyroid follicles bound to thyroglobuline. Thyroid hormones are released by proteolysis. In the peripheral blood, T₄ is converted to T₃. Antithyroid drugs act by inhibiting the thyroid peroxidase-mediated formation of T₃ and T₄ and compete with iodothyronine residues for oxidized iodine. Moreover, they inhibit iodine oxidation. Propylthiouracil (PTU), but not methyl-mercaptoimidazole (MMI), inhibits the monodeiodination of thyroxine to triiodothyronine.

Pharmacokinetics

Absorption of MMI from the gastrointestinal tract occurs rapidly and almost completely. Peak serum concentrations increase linearly and are in the range of 300 ng/ml 1–2 h after oral ingestion of 15 mg MMI. In vitro carbimazole is an effective inhibitor of iodination without prior hydrolysis to MMI. In contrast, carbimazole itself is inactive in vivo. During absorption and in serum it is almost completely converted to methimazole. Ten milligram carbimazole is equivalent to 6.7 mg MMI [2]. MMI is virtually not protein bound. The total volume distribution is about 40 L. The serum half life is 4–6 h and remains unchanged in hyperthyroid patients. Patients with hepatic disease have prolonged plasma disappearance, whereas in kidney disease the metabolism is unchanged. Because of its lipophilic character the transplacental passage and excretion in breast milk is high [1]. Little MMI is excreted in urine. Little is known about the products of metabolism of MMI and their way of excretion [1]. MMI can be applied parenterally.

PTU is also well absorbed from the gastrointestinal tract. Peak serum concentrations are in the range of 3 µg/ml at 1 h after drug ingestion after an oral dose of

150 mg. PTU, 80–90%, is protein bound. The total volume distribution is around 30 L for PTU. The serum half life of PTU is 75 min. It is not altered in patients with thyrotoxicosis, renal disease and, in contrast to MMI, in liver disease. PTU is mostly excreted in the urine after hepatic conjugation with glucuronide [1]. Biotransformation of PTU primarily occurs at the S group. It results in substantial loss of antiperoxidase activity. The metabolites, 6-*n*-propyluracil, *S*-methyl-PTU, PTU disulfide and PTU glucuronide, are only weakly active or completely inactive as thyroid peroxidase inhibitors. Because of its high protein binding and ionization at a physiologic pH, PTU is excreted in breast milk to a lesser degree than MMI. PTU crosses the placenta to an equal extent as MMI.

Both drugs, MMI and PTU, are actively concentrated by the thyroid gland. Intrathyroidal concentrations of MMI are in the range of 5×10^5 M. There is no difference in intrathyroidal concentrations of MMI 3–6 and 17–20 h after ingestion of 10 mg of carbimazole. Little is known about intrathyroidal concentrations of PTU. Eight hours after a single dose of 10 mg of MMI or 100 mg of PTU inhibition of intrathyroidal organification of iodide is about 90% and 60%,

respectively. This may be one reason for the longer effect of MMI compared with PTU. MMI can be administered once daily, whereas PTU should be applied 3 times a day.

Metabolism of the drugs by TPO is largely iodine dependent. Under conditions of reversible inhibition of iodination, the drugs are rapidly metabolized to higher oxidation products such as sulfonate and sulfate, with disulfide as an intermediate. If there is irreversible inhibition of iodination (higher drug to iodide ratio), some of the drug is oxidized only to the disulfide stage, but the TPO is simultaneously inactivated and no iodination is observed [2].

Clinical Use (Including Side Effects)

In patients with a first episode of ►Graves' disease, thionamides are used for long-term treatment to achieve remission of the organ-specific autoimmune disease. The standard therapy in Europe is a 1–1.5 year course of antithyroid drug treatment. In contrast, ►radioiodine is the preferred initial treatment in North America [2, 3, 5]. The relapse rate following antithyroid drug treatment is approximately 50% within 1–2 years. Most relapses occur within the first 12 months [2, 3]. After an unsuccessful course of antithyroid drug treatment there is little chance that a second course will result in permanent remission [2, 3]. Therefore a definite treatment, i.e. surgery or radioiodine, should be performed in these cases. Various parameters have been tested for their ability to predict the outcome of the individual patient after withdrawal of antithyroid drug therapy. A prospective study showed a greater chance of remission in patients with mild hyperthyroidism, smaller goiters, a lower base-line level of antithyrotropin-receptor antibodies. However, until present no reliable markers with predictive statistical significance for the individual patient's remission or relapse has been identified [2]. In ►thyroid autonomy, which is mainly caused by somatic ►TSH receptor or Gs α mutations [3, 5], a spontaneous remission (e.g. by nodule apoplexia) is very uncommon. Therefore, antithyroid drug treatment is only used to render patients euthyroid before ablative treatment [2, 5].

Initial daily doses of 10–40 and 100–600 mg are recommended in clinical practice for MMI and PTU, respectively [1, 2]. Several studies have shown that treatment of hyperthyroidism with single daily doses of 10–40 mg of MMI is effective in the induction of euthyroidism in 80–90% of patients within 6 weeks [2]. The aim of the further antithyroid therapy is to maintain euthyroidism with the lowest necessary drug dose. Intrathyroidal drug accumulation is one cause for the efficiency of a single daily dose regimen. Moreover, a once daily dose yields better patients' compliance. Single daily doses of PTU have been shown to be less effective in achieving euthyroidism than administration of three divided doses a day. If a once daily

dose regimen is considered for the treatment of hyperthyroidism, MMI is preferred to PTU [1, 2]. In the case of antithyroid drug therapy before radioiodine therapy MMI should be preferred to PTU, because PTU increases the failure of radioiodine therapy, which may be related to its ability to neutralize iodinated free radicals produced by radiation exposure [1]. This effect can be overcome by increasing the radioiodine dose. Continuous application of MMI during and until 4 weeks after radioiodine therapy with the aim to maintain euthyroidism until the effect of the ¹³¹I therapy sets in was shown to reduce the final cure rate. It should therefore be discontinued a few days before radioiodine therapy and resumed after approximately 1 week.

The response to thionamides depends on the dose and the iodine intake. It occurs faster in subjects living in countries with moderately low iodine intake than in areas with iodine sufficiency [2]. Antithyroid drug doses should be gradually decreased to the minimal maintenance dose as the serum thyroid hormone levels fall. The aim is to restore the euthyroid state within 1–2 months. Relapses of Graves' hyperthyroidism usually occurs within the first 3–6 months after discontinuation of antithyroid drugs. A lifelong follow-up is necessary to prevent spontaneous hypothyroidism, which can develop years after thionamide therapy of Graves' disease [1]. The "block-and-replace regimen" with the simultaneous administration of an antithyroid drug and L-thyroxine is used in case of poor patients compliance or if follow-up is difficult [3]. The titration regimen with low dose therapy showed fewer adverse effects than the high dose "block-and-replace regimen" and was not less effective.

MMI and PTU can lead to methimazole embryopathy with choanal or esophageal atresia. In pregnant women the antithyroid drug dose should be minimized to prevent fetal hypothyroidism by maintaining the maternal free thyroxine serum level slightly above the upper limit of normal.

Side Effects

Antithyroid drugs have several side effects. The most frequent side effects are maculopapular rashes, pruritus, urticaria, fever, arthralgia and swelling of the joints. They occur in 1–5% of patients [1, 2]. Loss of scalp hair, gastrointestinal problems, elevations of bone isoenzyme of alkaline phosphatase and abnormalities of taste and smell are less common. The incidence of all these untoward reactions is similar with MMI and PTU. Side effects of MMI are dose-related, whereas those of PTU are less clearly related to dose [1]. PTU may cause slight transient increases of serum aminotransferase and γ -glutamyl transpeptidase concentrations but also severe hepatotoxicity whereas methimazole or carbimazole can be associated with cholestasis. The side

Antitrypanosomal Drugs. Table 1 Different treatment strategies depending on the cause of hyperthyroidism

	Graves disease	Hot nodule and toxic multinodular goiter Etiology
	Organ specific autoimmune disease	Constitutively activating somatic mutation in TSH receptor or in Gs α
	TSH receptor antibody production	
Remission after antithyroid drug treatment	In 40–50% of patients 1 year after treatment withdrawal	No remission
Antithyroid drug treatment	1 year	Until euthyroid
Treatment with radioiodine or surgery	If relapse after 1 year of antithyroid drug treatment	After euthyroidism is achieved

effects usually appear within the first weeks or months after starting treatment. They occur more frequently with higher drug doses [1, 2]. If they are severe enough to alter treatment, it is possible to change from MMI to PTU or vice versa, although there is an estimated cross sensitivity of about 50% [2]. Most of the minor side effects are considered to be allergic reactions. Serious side effects such as bone marrow-depression, vasculitis, systemic lupus-like syndrome, cholestatic jaundice, hepatitis, hypoglycemia due to antiinsulin antibodies and hypoprothrombinemia are rare. They occur in approximately 0.2–0.5% of patients [1]. Depression of the bone marrow mostly appears as agranulocytosis, but also aplastic anemia and thrombocytopenia can be found. Symptoms of agranulocytosis like sore throat, fever and stomatitis are rare. Because of the sudden onset of agranulocytosis it can mostly not be detected in time by the routine leucocyte cell count. It occurs mostly within the first 3 months of treatment [1, 2]. Therefore, patients should be advised to stop taking the drug immediately if sore throat, pharyngitis or fever occur, and immediately seek medical attention and an urgent blood cell count. Most patients recover from agranulocytosis after discontinuation of antithyroid drugs. But deaths have been reported in 20% of patients despite treatment with intravenous broad spectrum antibiotics. Granulocyte colony-stimulating factor has been administered, but did not yield a better outcome in the treatment of antithyroid drug-induced agranulocytosis compared with antibiotic therapy only, whereas the time of recovery may be shortened by G-CSF therapy. The risk of agranulocytosis is greater in patients given larger doses and in older patients [1, 2]. Vasculitis and lupus-like syndrome occur much more frequently with PTU than with MMI. Treatment consists of discontinuation of the drug and the use of high doses of glucocorticoids. Major side effects usually occur within the first 3 months after the start of antithyroid drug treatment but can also appear during prolonged treatment and after reinstitution of the drug [1, 2, 3].

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Antitrypanosomal Drugs

The etiological agents of the African sleeping sickness are *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. Despite the enthusiasm shown by Robert Koch and Paul Ehrlich progress in the control of this disease achieved since the 19th century is sobering. Only four substances have been introduced, in total, in the therapy of African sleeping sickness, although the incidence of this disease has increased tremendously during the past decades with an estimated 50 million people at risk of infection and approximately 20 000 new cases reported each year. Suramin, the oldest of the antitrypanosomal drugs, was discovered by Paul Ehrlich and introduced in 1922. It was followed by pentamidine in 1937. Both drugs are only effective against early stages of the disease. The first and only drug effective against cerebral stages of African trypanosomiasis, of both *T. brucei gambiense* and *T. brucei rhodesiense* type, is melarsoprol, which has been in use for more than 50 years. It is still the drug of choice in most endemic areas despite its marked tendency to induce reactive encephalitis. Since then, drug and vaccine development in this field has declined to near zero. The only new antitrypanosomal substance so far has been eflornithine (difluoromethylornithine), which was introduced in 1990. It was originally developed for cancer therapy and proved later to be effective against intracerebral *T. b. gambiense*.

Nowadays, treatment of African sleeping sickness with the prevailing drugs faces three major problems:

(1) severe side effects, especially of melarsoprol; (2) wide spread drug resistance; and (3) lack of interest in drug development and production due to a low return of investment. To make matters worse, in recent years the producers of melarsoprol as well as eflornithine have tried to stop the production of these drugs entirely. A vaccine against trypanosomiasis is also not in sight, and efforts are complicated by the parasite's ability to constantly change the antigenic properties of its surface coat, a phenomenon called antigenic variation.

► Antiprotozoal Drugs

Antituberculosis Drugs

Antituberculosis drugs or antimycobacterial agents are specifically used for the treatment of tuberculosis (*Mycobacterium tuberculosis* infections). First-line drugs in tuberculosis therapy are isoniazid, rifampicin, ethambutol, pyrazinamide and streptomycin (Ribosomal Protein Synthesis Inhibitors). In order to minimize the development of drug resistance, a compound drug therapy is employed. In a first phase of two months, a combination of three drugs is employed. In a second phase of about four months, a combination of two drugs is used. A major problem is the increasing resistance of *Mycobacterium tuberculosis* strains against the first-line drugs. Infections caused by resistant strains are treated with combinations of second-line agents (e.g. capreomycin and cycloserine).

Antitumor Drugs

► Antineoplastic Agents

Antitussive Drugs

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Definitions

► **Cough** is an essential protective reflex response to irritating stimuli in the respiratory tract. It involves the sudden, usually involuntary, expulsion of air from

the lungs. It can prevent foreign bodies from entering the lungs, or aid the removal of mucus and irritants from the lungs. Cough is a common symptom in upper respiratory tract infections and more chronically in asthma, chronic obstructive pulmonary disease (COPD) and lung cancer or may also indicate some other underlying disorders such as gastro-oesophageal reflux disease. It is also frequently caused by smoking. Conversely, in certain conditions such as Parkinson's disease, stroke and motoneurone disease, cough may be severely affected and the patient loses this protective function. Cough is useful if it is aiding the expulsion of foreign substances, but chronic cough is an unpleasant, difficult symptom for patients to live with and maybe debilitating.

Basic Mechanisms

Physically, cough results from a series of events starting with the patient inhaling, often to near maximum levels. Secondly, the glottis closes and the breathing muscles cause compression of the air in the lungs, leading to high pressure in the pleural space and alveoli. The expulsive phase occurs when the glottis reopens allowing the compressed air to escape. It is at this point that the cough sound is heard, and also that foreign materials which may have been deposited in the lungs, are removed. Coughing often occurs in bouts, or epochs, rather than as discrete events. *Cough* may be voluntary or involuntary – sub threshold stimuli may evoke the urge to cough, but not the reflex itself.

Airway irritation is detected by ► **sensory nerves** which are found within the walls of the airways. Various nerve endings are found in this area, but it is thought that the ► **rapidly adapting receptors (RARs)** are the predominant sensors of *cough*. RARs rapidly adapt to a maintained stimulus, and have thin myelinated A δ fibres. Slowly adapting receptors and C-fibres (C-fibres are sensory nerves with unmyelinated axons and can have a modulatory role on the cough reflex.) can have a modulatory role on the cough reflex. Several different types of sensor have been found, and more than one type of fibre is involved – it is possible that the combination of fibres and the nature of the discharge regulate *cough* as much as the quantity of action potentials.

These primary afferents project from the airway into the CNS exclusively via the vagus nerve, via the nodose or jugular ganglia. These neurons enter the brainstem and terminate in the nucleus tractus solitarius. Here they synapse with other neurons which determine the various motor components of *cough*. At these central synapses, there are many neurotransmitter receptors present (e.g. tachykinins, glutamate, 5-HT, GABA, NMDA, dopamine, opioids and nociceptin), which may be the site of action of centrally acting antitussive drugs (see below). It is also here that other sensory afferents terminate, and may modulate the synaptic signalling. If there are disorders in other organs which also have

sensory neurons carried in the vagus, it is here that these nerves may interact with airway neurons to cause *cough*. In contrast, in diseases where *cough* is reduced, it is generally due to problems in the efferent part of the reflex loop.

Previously it was thought that there was a central “cough centre” in the CNS, but current research suggests that this is an over-simplification. The ability to coordinate a combination of a variety of motor outputs is essential for *cough*. For example, outputs go to control airway structures such as the glottis and a wide range of breathing muscles such as the diaphragm and intercostal muscles. In addition, synchronisation with the respiratory rhythm is required as it is not possible to *cough* and breathe concurrently. Finally, the CNS is thought to contain a gating mechanism by which the brain determines whether the arriving stimuli are of sufficient magnitude for a cough to occur. The cortex is not necessary for cough, but it can exert descending control on the reflex. (Fig. 1)

The sensory nervous system which governs cough is subject to plasticity (►[nerve plasticity](#)) – such that there

may be an enhancement of this cough pathway, either by changes in the receptors on ►[sensory nerve](#) endings, the ganglia or within the CNS which can increase the activity of these neurons leading to a hypertussive state.

Pharmacological Interventions

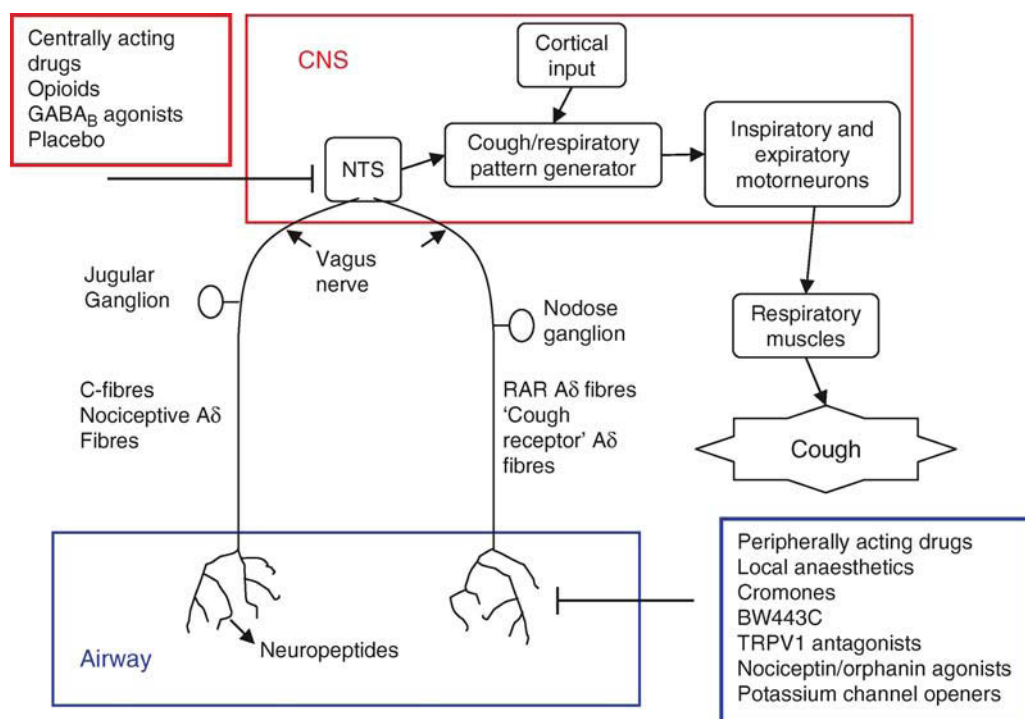
Cough is currently a huge unmet clinical need, as none of the currently available treatments are reliably effective [2]. However, there are many treatments which are currently used, with variable levels of success. In addition, if it is caused by another condition, such as gastro-oesophageal reflux disease, then treatment of that may reduce cough.

Drugs

Centrally Acting Drugs

Opioids

Opioids (►[opioid systems](#)) are thought to exert their antitussive effects by acting as agonists at μ - and κ -opioid receptors in the CNS. Activation of these receptors activates various G-proteins and leads to the inhibition of



Antitussive Drugs. Figure 1 Schematic diagram of the cough reflex and sites of action of some tussive agents. Airway sensory nerves activated in response to a tussive stimulus travel through the vagus nerve to the medulla, where they terminate in the nucleus tractus solitarius (NTS). Second order neurons relay the message to the respiratory pattern generator, which modifies the activity of the inspiratory and expiratory motoneurons and leads to cough. Tussive agents can activate a variety of airway receptors to cause cough. Many nerve types (both peripherally and centrally) undergo phenotypic changes and have increased responses to tussive stimuli following inflammation. The sites of action of antitussive drugs are shown. Abbreviations: RAR, rapidly adapting receptor; CNS, central nervous system. Figure adapted from [1].

the activity of most neurons, but the activity of a few are increased. Recent evidence suggests that morphine may also interact with ►TRPV1 receptors to explain some of its antitussive activity.

Centrally acting drugs include dextromethorphan and codeine. However the possibility that there may also be peripheral effects of these drugs has led to the development of BW443C (see below).

Opioid drugs are often more effective than other non-narcotic treatments, but they are also associated with more side effects making them less suitable for many patients. Higher doses which are more effective are also associated with undesirable effects such as sedation.

Peripherally Acting Drugs

Local Anaesthetics

Local anaesthetics are more consistently effective than other therapies, but their use is controversial. High concentrations are needed for therapeutic benefit, but this also increases the amount crossing the blood brain barrier and entering the brain producing unwanted effects. Topical administration to the airways can reduce this.

Patients who do not obtain sufficient symptomatic relief from other treatments may use lidocaine, benzonatate, bupivacaine topically or mexiletine orally.

Tachykinin Antagonists (►Tachykinins)

►C-fibre afferents from the airways contain peptide tachykinin transmitters such as substance P (SP) and neurokinins A and B (NKA and NKB). Stimulation of these nerves can also cause local release of these mediators at their peripheral terminal, allowing them to enhance the activity of the *RARs*. SP, NKA and NKB act at the tachykinin receptors (NK₁–NK₃), and so understandably, antagonists for NK₂ in particular appear promising in cough.

Cromones

Both disodium cromoglycate and nedocromil sodium have antitussive effects in humans, particularly against ACE inhibitor induced cough. This suggests an effect on bradykinin induced changes in ►sensory nerve function. Antitussive activity of these drugs is thought to occur by increasing the depolarisation of sensory nerves, which increases the threshold for an action potential and therefore inhibits the activity of these neurons.

GABA_B Receptor Agonists

►GABA is the predominant inhibitory neurotransmitter in the CNS. Baclofen acts centrally as an agonist at the GABA_B receptor, which increases inhibition of nerves. 3-Aminopropylphosphinic acid (3-APPi) has been shown experimentally to act as an antitussive at peripheral nerves and preclinical evidence suggests that baclofen indeed has antitussive actions clinically [3].

Novel Drugs

BW443C

BW443C is a novel opioid used for the treatment of cough, but which does not enter the brain and so exerts its effects only on peripheral nerves. It has not been tested as an antitussive in humans due to its rapid metabolism in the lungs but the concept of a peripheral opioid is still possible.

Nociceptin/Orphanin Receptor Agonists

Nociceptin and orphanin are synonyms for the peptide that acts at an opioid-like receptor. Nociceptin may act by inhibiting tachykinin release from sensory ►C-fibres, and a clinical trial has started to test its effects on cough.

TRPV1 Receptor Antagonists

Capsaicin, the pungent chemical from chilli peppers, induces cough. Capsaicin is an agonist at the transient receptor potential vanilloid receptor 1 (TRPV1) – a polymodal receptor which integrates several harmful stimuli such as noxious heat, low pH and various possible endogenous mediators to mediate pain and cough. TRPV1 receptors have been demonstrated to be upregulated in patients with chronic cough [4].

Capsaicin is used to cause cough experimentally and clinically and the TRPV1 antagonist capsazepine can inhibit cough elicited by both capsaicin and citric acid. This suggests that other TRPV1 antagonists could be effective treatments for cough (recently reviewed in [5]). However, capsazepine does not block hypertonic saline induced cough, suggesting that other tussive receptors are also important [5].

Potassium Channel Openers

An effect of opening ►K⁺ channels is to hyperpolarise the primary sensory neurons. Similarly to local anaesthetics, this makes the cell less likely to produce an action potential because more depolarising stimuli are needed to overcome the block. NS1619 is an example of this type of drug which has initially shown antitussive activity in a variety of experimental systems.

Quaternary Ammonium Salts

Quaternary ammonium salts such as carcamium chloride (RSD 931) have been shown to be antitussive whilst having much reduced local anaesthetic activity. Whilst the molecular mechanisms underlying this antitussive activity is not understood, RSD 931 appears to be Aδ fibre selective and may represent a novel class of antitussive drug. More recently JMF2-1 a lidocaine derivative that blocks Na⁺ channels has had beneficial effects in the airways without significant local anaesthetic activity.

►Opioid System

►TRP Channels

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Antiviral Drugs

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Synonyms

Antivirals; Virostatics

Definition

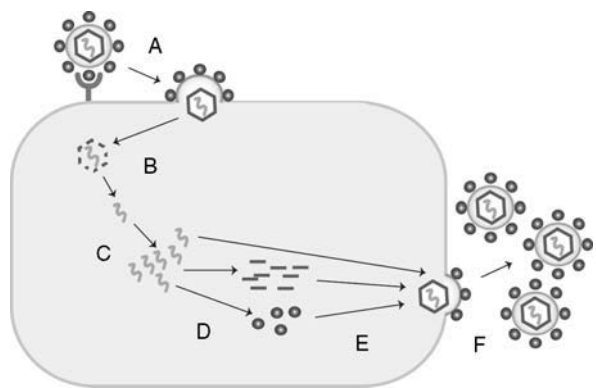
Viruses are small infectious agents composed of a nucleic acid genome (DNA or RNA) encased by structural proteins and in some cases a lipid ►envelope. They are the causative agents of a number of human infectious diseases, the most important for public health today being acquired immunodeficiency syndrome ►(AIDS), hepatitis, influenza, measles, and viruses causing diarrhoea (e.g., rotavirus). In addition, certain viruses contribute to the development of cancer. Antiviral drugs inhibit viral replication by specifically targeting viral enzymes or functions and are used to treat specific virus-associated diseases.

Mechanism of Action

Basic Principles

Viruses are obligatory intracellular parasites that can only replicate within an appropriate host cell. They rely on host cell-derived factors and mechanisms, and encode only few enzymes and other proteins of their own. Consequently, interfering with virus replication without inflicting damage to the host requires highly specific approaches and there is no general mechanism of action for virostatics. In contrast to antibacterial agents, where fundamental differences between prokaryotic and human cells (e.g., bacterial cell wall synthesis) can be exploited to inhibit a broad range of

bacteria without significant toxicity to the patient, antiviral drugs are mostly highly selective for a specific virus or a limited number of related viruses. Furthermore, many human virus infections are acute and are rapidly controlled by the immune system. In these cases, the highest ►viremia – i.e., the time when the patient would benefit the most from causative antiviral treatment – usually precedes the clinical manifestation of symptoms and the diagnosis by serological methods. For these reasons, the number of currently available antiviral drugs is low compared to chemotherapeutics effective against other classes of infectious agents. Most antivirals have been developed to control chronic or recurrent virus infections, and in many cases antiviral treatment does not result in elimination of the virus but rather reduction of virus replication and alleviation of symptoms. In principle, antivirals can be targeted at any of the viral replication steps outlined in Fig. 1. (A) Binding: Viruses attach to their host cells via binding of a viral surface protein to one or several receptor molecule(s) on the plasma membrane. This interaction is highly specific and presents a very attractive target for antiviral intervention. The most advanced compounds in this class are the human immunodeficiency virus (HIV) coreceptor antagonists. These substances bind to and block the cellular chemokine receptors CCR5 or CXCR4, respectively, which are required in addition to the CD4 receptor to mediate HIV entry. The most promising results so far have been obtained with CCR5 antagonists, and one of these compounds (maraviroc) has recently been approved for treatment. (B) Entry of the virus into the cell and release of the viral genome (uncoating): The drug enfuvirtide targets the HIV entry process, by preventing the fusion of the virus with the plasma membrane. Although the uncoating process is not characterized in its entirety for most viruses, random screening has identified amantadine/rimantadine, which



Antiviral Drugs. Figure 1 Basic steps of viral replication: (A) binding, (B) entry, (C) genome replication, (D) gene expression, (E) assembly, (F) release.

inhibit this step in influenza A replication. In addition, uncoating inhibitors specific for entero- and **picornaviruses** are under development. (C) Replication of the viral genome: Viruses contain either single- or double-stranded DNA or RNA genomes and employ a variety of replication strategies. Thus, there is no general mechanism or replication enzyme common to viruses. However, to accommodate for various replication strategies, many viruses encode their own polymerase(s), the biochemical and structural properties of which differ in some respects from those of the host cell polymerases. This can be exploited to specifically interfere with viral genome replication, and a number of inhibitors targeting viral replicases have been developed as antiviral drugs. (D) Expression of viral genes leads to the production of virus proteins: The basic machineries for transcription of virus genes and translation of mRNA into viral proteins largely rely on cellular factors and are therefore difficult to target. Many viruses encode proteins regulating transcription or mRNA transport and modification (e.g., the HIV Tat and Rev proteins), which are potential candidates for inhibition. However, drugs specifically interfering with viral gene expression have not yet been developed. (E) Virus assembly comprises transport of the virion components to the assembly site, formation of an ordered capsid structure and in some cases morphological maturation of the particle into a fully infectious state. Capsid stability depends on multiple interactions between viral structural proteins, and interfering with only a few interactions should suffice to disturb the ordered capsid architecture essential for infectivity. The subunit interfaces, unlike viral enzymes, do not have correlates in the host cell. This should allow the design of efficient and specific inhibitors, but to date insufficient understanding of the molecular processes involved and the lack of suitable assay systems has prevented the development of assembly inhibitors for antiviral therapy. However, highly effective antiviral drugs targeting the process of virion maturation (**protease** inhibitors) have been developed against HIV. (F) Virus release from the producing cell is required for virus spread: It can be accomplished by cell lysis, exocytosis or, in the case of many enveloped viruses, by budding from the plasma membrane. Virus budding is a complex process involving a number of host cell-derived factors, and drugs targeting the budding process itself have not yet been developed. However, the recently introduced influenza neuraminidase inhibitors are effective by blocking the release and spread of influenza viruses.

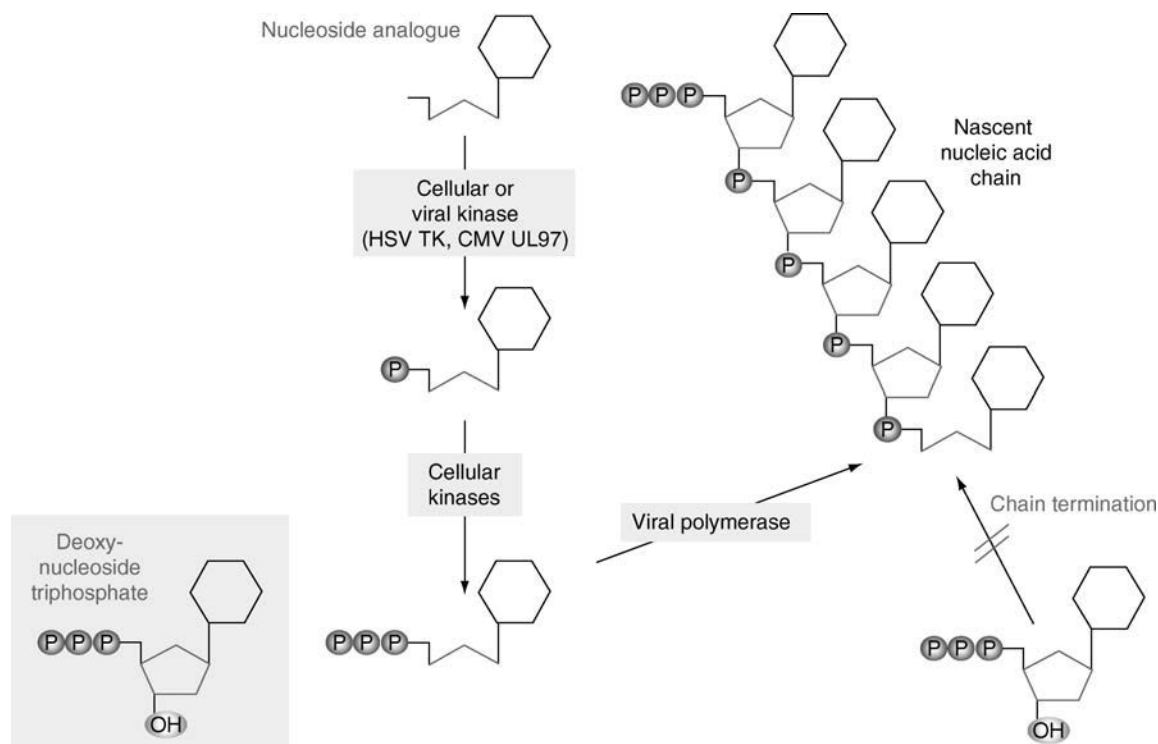
Interferon (IFN) differs from *bona fide* antiviral drugs since it is a natural defense protein of the host organism and does not directly interfere with the viral replication steps. Interferons are small glycoproteins inducing immune modulatory and antiviral activities. They are secreted by lymphocytes, leukocytes and fibroblasts in response to foreign nucleic acids (dsRNA).

IFNs are classified into three groups α , β , and γ , and the different classes are produced from different cell types. Recombinant IFN- α is used in the treatment of chronic hepatitis B and C.

Mechanisms of Action of Currently Used Antiviral Drugs

The initial steps in viral replication are attachment to and entry into the host cell. One drug targeting the fusion event of the virus with its host cell is approved for clinical use against HIV: The peptide derivative enfuvirtide binds to a helix in the viral envelope protein gp41, which is exposed upon binding of HIV to its cellular receptor. This binding of enfuvirtide blocks a conformational rearrangement of gp41 molecules which is required to mediate the fusion of viral and cellular lipid membranes. Similar compounds with improved potency and pharmacokinetics have been generated, but are not approved for clinical use. Several drugs inhibiting the binding of HIV by inhibiting the interaction of the virus with cellular receptor or coreceptors are currently under preclinical and clinical development. One HIV-1 co-receptor antagonist (maraviroc) has recently been approved. Available drugs acting at a step in influenza virus replication are the adamantane derivatives, amantadine and rimantadine. Amantadine has been in clinical use since the early 1970s, but its mechanism of action was elucidated only 20 years later. It blocks the M2 ion channel in the envelope of influenza A virus, thereby inhibiting virus uncoating. Adamantanes are not efficient against influenza B, which lacks the M2 protein. Entry inhibitors specific for entero- and rhinoviruses are drugs based on the substance pleconaril. It binds to a hydrophobic pocket on the surface of picornavirus capsids resulting in conformational changes of the **capsid** that interfere with the release of the viral RNA genome into the host cell. Pleconaril is tested in clinical trials but is currently not approved for therapy.

Polymerase inhibitors with different mechanisms of action account for the largest group of currently available antiviral drugs. The most important class are the chain terminating nucleoside analogues (Fig. 2). The prototype of this class is acyclovir (ACV) which is used against herpes simplex (HSV) and **varicella zoster viruses** (VZV). ACV is an acyclic analogue of the nucleoside thymidine, with carbon atoms C2 and C3 missing from the deoxyribose ring. Phosphorylation by HSV thymidine kinase (TK) inside an infected cell yields ACV-monophosphate, which is further converted into ACV-triphosphate by cellular enzymes and then serves as a substrate for the HSV polymerase. ACV incorporation into DNA results in chain termination due to the lack of the 3'OH group required for further elongation. Since both monophosphorylation and incorporation into DNA are preferably carried out by



Antiviral Drugs. Figure 2 Mechanism of action of chain-terminating nucleoside analogues.

viral enzymes, selectivity on two levels reduces toxicity to uninfected cells. This principle is also exploited by related drugs acting against herpesviruses (brivudin, famciclovir). Efficacy of phosphorylation and incorporation into DNA are not correlated: whereas penciclovir (oral prodrug: famciclovir) is a much better substrate for TK than ACV, penciclovir triphosphate is incorporated less efficiently by the viral polymerase than ACV-triphosphate. An optimal inhibitor should be a good substrate for both viral enzymes. Human cytomegalovirus (HCMV) lacks a *tk* gene and is relatively insensitive to ACV, but a protein encoded by gene *UL97* of HCMV is able to phosphorylate the nucleoside analogue ganciclovir (GCV). This analogue also efficiently interferes with cellular DNA polymerisation, and thus has a higher toxicity than ACV. Resistance against GCV can develop through mutations in either *UL97* (90%) or the viral polymerase gene. The alternative drug cidofovir is a cytosine phosphonate analogue, which only depends on cellular enzymes for its conversion into the active form. Thus, its efficacy is not affected by mutations in *UL97*. A direct inhibitor of viral polymerase, which does not require intracellular activation, is foscarnet (phosphonoformic acid). The chain terminating mechanism is shared by another important group of antivirals, the nucleosidic and nucleotidic **reverse transcriptase** (RT) inhibitors (NRTI and NtRTI), which inhibit the RT of HIV. A number of different NRTIs are available in different

formulations, e.g., azidothymidine (AZT), ddI, ddC, and d4T. All of these are nucleoside analogues, in which the 3'OH group is missing or replaced by another functional group, e.g., an azido group in AZT. NRTI are also applied as prodrugs which have to be phosphorylated by cellular kinases into their active triphosphate form. NtRTI (Tenofovir, Adefovir) are monophosphorylated derivatives. In this case the first- and often rate-limiting step of activation is circumvented. Hepatitis B virus (HBV) (**Hepatitis**), another important human pathogen, also encodes an RT, and several nucleoside/nucleotide analogues originally developed against HIV (e.g., lamivudine) are also active against HBV. The compounds lamivudine, adefovir and the HBV polymerase selective nucleoside derivative entecavir are available as anti-HBV drugs, and several other drugs from this class are expected to be approved for this use in the future (e.g., telbivudine, clevudine, emtricitabine). As obvious from this example, chain terminators are not exclusively selective for the particular viral polymerase targeted, since nucleoside analogues bind to the relatively conserved active site of polymerases. Thus, a certain degree of inhibition of cellular polymerases also has to be taken into account. Furthermore, soon after the introduction of AZT for the treatment of AIDS patients in 1987, it became apparent that although it was possible to lower the viral load by up to 80% through AZT monotherapy, the therapeutic success was limited by the rapid emergence of drug resistant virus. The

treatment is possible using idoxuridine or vidarabin. Foscarnet can be used against resistant viruses but has a higher risk of side effects (nephrotoxicity). Indications for the use of GCV or valganciclovir are ►CMV chorioretinitis in ►AIDS patients and CMV colitis. It is also used to prevent interstitial pneumonia in immunosuppressed patients. GCV is more toxic than ACV and causes neutropenia in about 40% of patients. Alternatively, CMV infection can be treated with cidofovir or foscarnet, which are also effective against GCV-resistant CMV but have an even higher toxicity. In pharmacologically immunosuppressed transplant recipients, GCV or ACV are administered to prevent CMV or HSV disease, respectively. An anti-CMV drug based on a new mechanism of action is Fomivirsen. It is an antisense RNA which inhibits the synthesis of a viral protein and is approved for intravitreal treatment of CMV retinitis.

A combination therapy of IFN- α ("pegylated", i.e., coupled to polyethylene glycol, which prevents its rapid clearance from the body) and ribavirin over 24–48 weeks is the most effective way to treat chronic hepatitis C. Therapeutic success depends on virus genotype and viral load, but overall this treatment eliminates the virus in about 60% of patients. A major side effect of this combination therapy is hemolytic anemia, attributed to ribavirin. Interferon monotherapy can be used as postexposure prophylaxis after accidental exposure to HCV to prevent chronification. Patients with chronic hepatitis B, characterized by elevated serum alanine aminotransferase levels and detectable HBe antigen for >6 months, are currently treated with pegylated IFN- α alone as the first choice. Clinical improvement can be accomplished in about 30–40% of treated patients. Fatigue, muscle aches, headache, nausea and diarrhea are common side effects of interferon. The most common serious side effect is depression. Treatment with antiviral nucleoside analogues against HBV is also efficient as monotherapy and is used mainly in cases with moderate elevation of ALT levels and when use of interferon is contraindicated (e.g., for patients with liver cirrhosis). Resistance development occurs frequently upon prolonged treatment with lamivudine; in this case, treatment can be switched to adefovir. The newest drug from this class, entecavir, appears to be superior to lamivudine with respect to both efficacy and resistance development. Combination of interferon with nucleoside analogues or combination of two nucleoside analogues has not been found to have advantageous effects. In the case of acute hepatitis B, antiviral treatment is not recommended.

The broadest spectrum of antiviral drugs is available against HIV. However, monotherapy with any of these drugs leads to rapid treatment failure due to selection and further evolution of resistant viruses. Since acquisition of resistance mutations requires virus replication,

an efficient therapy regimen and patient compliance are paramount to minimize resistance development. Currently, AIDS patients in industrial countries are treated with a combination therapy known as HAART (highly active antiretroviral therapy) involving at least three different anti-HIV drugs from the classes mentioned above (NRTI, NNRTI, PRI, entry inhibitor). Treatment is currently indicated when the patient shows symptoms of AIDS or has a CD4⁺ cell count below 200/ μ l. Treatment decisions in other cases are complex and need to be made on an individual basis. Triple therapy is also recommended as prophylaxis following accidental exposure to the virus. In this case, it is crucial that treatment is started immediately (best within 1–2 h). The introduction of HAART led to a significant decrease in AIDS morbidity and mortality. However, severe side effects (neutropenia, neurological problems, lipodystrophy) can occur especially under prolonged therapy, and even under HAART, resistant and multi-resistant viruses emerge and are transmitted. Thus, HAART requires selection of a suitable drug combination by a physician experienced in AIDS therapy, constant monitoring of viral load and individual adjustment in case of treatment failure or intolerable side effects. Successful treatment in the case of HIV infection does not mean eradication of the virus and therapy has to be continued over many years, probably life-long. For this reason, future goals do not only include the discovery of new anti-HIV drugs, but also the improvement of existing drugs in terms of galenics, side effects and possible combination formulations that make it easier to follow the therapy scheme. Finally, it should be noted that HAART is generally not available in developing countries, where most of the millions of HIV-infected people live.

High replication rates, error-prone polymerase as well as replication strategies favoring genetic recombination result in rapid virus evolution. Thus, the emergence of drug resistant variants is a general problem for antiviral therapy. Successful antiviral strategies will probably have to be combination therapies employing different drugs. Experiences indicate that cotreatment with two or even three drugs does not prevent viral resistance development, and broadening of the antiviral arsenal is a key issue. Future additions to this arsenal will result from different approaches: First, alternative and improved drugs based on the same mechanisms of action or on the same principles (e.g., inhibition of viral enzymes by substrate analogues) will be developed. Second, as the molecular understanding of viral biology increases and suitable assay systems for *in vitro* screening can be established, other steps of viral replication that are currently not accessible (assembly, transcriptional and posttranscriptional regulation or virus release) could become targets of chemotherapeutic intervention. Third, there are attempts to include new

and experimental therapeutic approaches, such as the specific shutdown of viral gene expression by antisense RNA or siRNA (small interfering RNA) or gene therapy, into antiviral strategies. As an alternative antiviral approach, all these strategies are currently considered mainly as potential inhibitors of HIV replication, but also discussed for development of novel treatments of chronic hepatitis B and C. While some encouraging results have been obtained in experimental settings and in small-scale clinical trials, many problems and questions regarding, e.g., specificity and durability of therapeutic effects, safe and targeted delivery of the antiviral principle, and viral escape by mutation remain to be solved before these can translate into clinical applications.

- ▶ Interferons
- ▶ Viral Proteases

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Anxiety

Anxiety is a normal reaction. Pathological anxiety interferes with daily-life activities and may be accompanied by autonomic symptoms (chest pain, dyspnoea and palpitations). Severe forms include phobic anxiety and panic disorder.

- ▶ Benzodiazepines

Anxiolytics

Anxiolytics are drugs used for the treatment of anxiety disorders. Apart from benzodiazepines, a frequently used anxiolytic is the 5HT_{1A} (serotonin) receptor agonist bupiron, which has no sedative, amnesic or muscle-relaxant side effects, but whose action takes about a week to develop. Furthermore, it is less efficacious than the benzodiazepines. Bupiron's mechanism of action is not fully understood.

- ▶ Anxiety
- ▶ Benzodiazepines

APCs

Antigen-presenting Cells.

- ▶ Immune Defense

Apelins and the Apelin Receptor

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Synonyms

Apelin receptor; APJ receptor; Angiotensin II receptor-like 1

Definition

The family of apelin peptides is derived from a single gene, activate a single G-protein-coupled receptor and are substrates for the ▶ [angiotensin converting enzyme-2 \(ACE2\)](#). Apelins regulate cardiovascular function and fluid homeostasis. The apelin receptor also functions as a co-receptor for infection of CD4-positive cells by human immunodeficiency virus (▶ [HIV](#)).

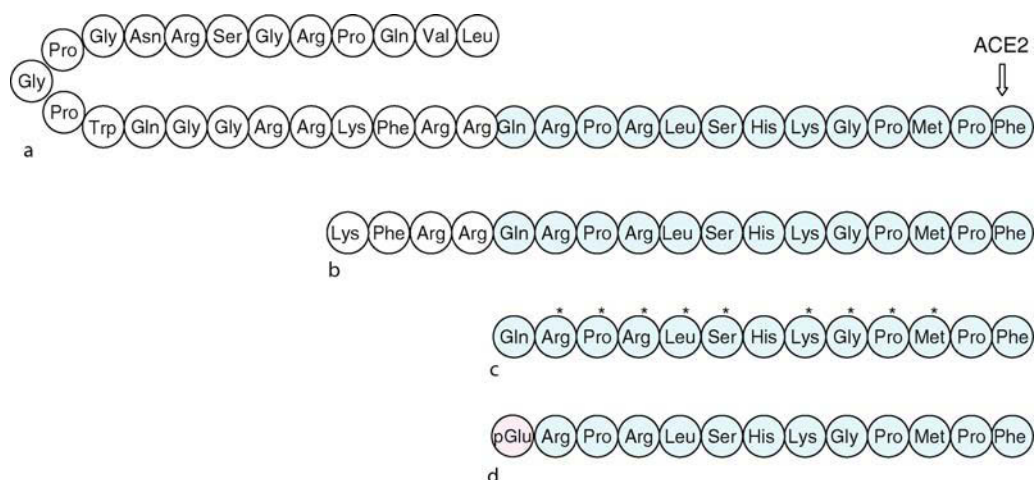
Basic Characteristics

The human gene encoding the putative APJ receptor was identified in 1993 by O'Dowd and colleagues using a degenerate PCR strategy as having a high degree of sequence similarity with the gene encoding the angiotensin AT₁ receptor (54% in the transmembrane spanning regions) [1]. However, angiotensin II did not activate APJ and it was therefore designated as an ▶ [‘orphan’ receptor](#). In 1988, apelin-36, (*APJ* endogenous *ligand*), a 36 amino acid peptide, was isolated from bovine stomach. Using the technique of reverse pharmacology, Tatemoto and colleagues found that the peptide bound and activated APJ receptors artificially expressed in cell lines [2]. APJ is now classified as the apelin receptor, in Family A of the superfamily of seven transmembrane spanning ▶ [G-protein-coupled receptors](#).

Apelin Peptides, Synthesis and Metabolism

Cloning of corresponding human and bovine cDNA led to the identification of a gene encoding a 77 amino acid precursor peptide, preproapelin, in a number of species (human, bovine, rat and mouse). This precursor contains a number of basic amino acid pairs (Arg–Arg or Arg–Lys) thought to be proteolytic cleavage sites for endopeptidases, resulting in a family of C-terminal peptides of varying sizes that can all activate the receptor (Fig. 1). Thus far, no specific enzymatic pathway has been discovered which is responsible for cleaving preproapelin into shorter mature peptides. Apelin-36 is highly conserved between species; the last 22 residues of the C-terminus are identical in mammals (Fig. 2). The endogenous forms detected in tissues and biological fluids (usually by chromatographic separation or gel filtration followed by immunoassay) comprise predominantly apelin-36, apelin-17, apelin-13 and following ▶posttranslational modification by

enzymatic conversion of the N-terminal glutamate to pyroglutamate, [Pyr¹]apelin-13 (Fig. 1) [3, 4]. This modification is common to a number of biologically active peptides, which confers resistance to degradation by peptidases. The relative abundance of these molecular forms varies according to tissue examined. For example, in the rat, peptides of a size close to apelin-36 were the major components in the lung, testis, and uterus, but both apelin-13 and apelin-36 were detected in the mammary gland. Apelin-13 predominated in plasma and brain (hypothalamus) with apelin-17 being detected. Apelin-12 also activates apelin receptors in a number of preparations being more potent than apelin-13 in reducing blood pressure in vivo in rats but apelin-12 has not been identified as an endogenous peptide. Apelin-16 has also been used in preparations. Apelin-11 and shorter sequences are devoid of binding activity to apelin receptors expressed in cells. Stepwise substitution of alanine for residues



Apelins and the Apelin Receptor. Figure 1 Amino acid sequences of (a) apelin-36 (b) apelin-17 (c) apelin-13 and (d), [Pyr¹]apelin-13. Amino acids identical in all peptides are shown in blue. The posttranslational modification of the N-terminal glutamate in apelin-13 to pyroglutamate, [Pyr¹] is shown in pink. ACE2 hydrolyses apelin-36 and apelin-13 resulting in the removal of the C-terminal residue. * indicates the residues found to be important for binding and activation of the apelin receptor by apelin-13].

	1	36
Human	LVQPRGSRNGPGPWQGGRRKFRRQRPRLSHGMPF	
Chimpanzee	LVQPRGSRNGPGPCQGGRRKFRRQRPRLSHGMPF	
Rat	LVKPRTSRTGPGAWQGGRRKFRRQRPRLSHGMPF	
Mouse	LVKPRTSRTGPGAWQGGRRKFRRQRPRLSHGMPF	
Bovine	LVQPRGPRSGPGPWQGGRRKFRRQRPRLSHGMPF	
<i>Xenopus laevis</i>	LVNPKMVRNSAPQRQANRRKLIQRPRLSHGMPF	
	** * *	* ** *

Apelins and the Apelin Receptor. Figure 2 Sequence alignment of mammalian and amphibian apelin-36 amino acid sequences. *Indicates residues conserved across all the species shown. Residues which differ from the human sequence are highlighted in red.

Arg-2, Pro-3, Arg-4, Leu-5, Ser-6, Lys-8 Gly-9, Pro-10 and Met-11 into apelin-13 reduced [125 I]-(Pyr¹)apelin-13 binding to apelin receptors expressed in cell lines, suggesting they are important residues for receptor interaction. Structure activity studies using fragments of apelin-17 also identified Gln-1, Pro-12 and Phe-13 not essential for apelin binding.

Metabolism of apelin peptides in vivo is not yet clear. ACE2, the closest human homologue of angiotensin converting enzyme, cleaves the C-terminal Phe of apelin-13 with high catalytic efficiency in vitro (Fig. 1). The enzyme is expressed in ►endothelial cells particularly in the heart and kidney. ACE2 also hydrolyses apelin-36 as well as other biologically active peptides including angiotensin II to angiotensin 1–7 (a functional antagonist of angiotensin-II which acts predominantly as a vasodilator) suggesting the apelin peptides may be part of the enzymatic cascade of the ►renin–angiotensin–aldosterone system in cardiovascular regulation. ACE2 is also a receptor for severe acute respiratory syndrome coronavirus (SARS CoV) [4].

Distribution of Apelin mRNA and Peptides

Apelin mRNA (Table 1) is present in a range of peripheral rat tissues including stomach, intestine, heart, liver, kidney, testis, ovary and adipose tissues with highest levels in the lung and in particular the mammary gland (Table 1). Highest mRNA expression in the rat brain included the spinal cord, olfactory tubercle, hippocampus, hypothalamus, pineal gland, pituitary and the cerebral cortex. Apelin-like immunoreactivity was detected in rat tissues including white adipose tissue, epithelial cells of the oxyntic stomach mucosa as well as in endothelial cells of small arteries and veins in mesenterium, omentum, heart, lung, gastrointestinal tract, spleen, pancreas and liver. In rat brain, immunoreactivity is present in neurones of the pons, medulla oblongata and arcuate nucleus and also in the supraoptic and paraventricular nucleus of the hypothalamus, where apelin positive neurones also expressed vasopressin mRNA. In humans, apelin-like immunoreactivity was detected in endocardial endothelial cells lining the atria and ventricles of the heart and endothelial

Apelins and the Apelin Receptor. Table 1 Distribution of mRNA encoding apelin peptides and receptor [4]

	Apelin receptor			Apelin peptides		
	Rat	Mouse	Human	Rat	Mouse	Human
Brain	++	+	+++	+	+++	++
Cerebellum	+		+	+		+
Pituitary	+		+	+		++
Spinal cord	+++	++	+	++		++
Adrenal gland	+			+		
Thyroid	++					
Spleen	–	+	+++	–	+	
Thymus		+	+		–	
Heart	++	+++	+	++	++	+
Endothelial cells	++					
Lung	+++	++	++	+++	++	+
Stomach	+		+	+		–
Small intestine	+		++	+		–
Large intestine	+		++	+		
Liver	+	+		–	–	–
Pancreas	–		+	–		+
Kidney	+	+	+	+	+	+
Testis	+	+	+	+	++	+
Prostate			+			+
Ovary	+	+	+	+	+	
Uterus	+	+	+	+	–	–
Placenta	++		++			+++
Mammary gland	+			++		
Skeletal muscle	++	++	+	+	+	–
Adipose tissue	++			+		
Cartilage	++					

cells lining large conduit vessels, small arteries and veins (<500 µm diameter) from lung, kidney and adrenal gland. Within endothelial cells, apelin-like immunoreactivity is present in secretory vesicles of the constitutive secretory pathway but not Weibel-Palade bodies of the regulated stimulated pathway, suggesting the peptide may be released from the former [4].

Apelin-like immunoreactivity has been quantified in tissues using antisera that mainly cross reacts with all apelin peptides. Levels in human atria were 650 pg/mg with lower but detectable levels in the ventricle (3 pg/mg). Apelin-like immunoreactivity in human plasma measured following extraction and radioimmunoassay ranges from 24–89 pg/ml, levels comparable to other locally acting endothelium derived peptides. This may reflect overspill from the vascular and endocardial endothelial cells, consistent with the proposed role as a locally released mediator. Whether there is any contribution from other sources such as epithelial cells of the gut, is unclear. Apelin levels rise in early heart failure and fall with severe disease although other studies reported no change in patients with dilated cardiomyopathy. Apelin is expressed and secreted by human and mouse adipocytes; with plasma apelin levels increasing with obesity and correlating with body mass index, suggesting a role as an adipokine.

In agreement with abundant mRNA in mammary gland, high levels of apelin are present in bovine colostrum; oral intake of apelin might modulate immune responses in neonates [4].

Distribution and Characterisation of Apelin Receptor

In the rat (Table 1), apelin receptor mRNA is widely distributed in almost all peripheral tissues (consistent with a vascular expression) with highest levels in lung and heart and lower levels in kidney, pituitary gland, ovary and skeletal muscle. In the rat CNS, apelin receptor is expressed in brain including the cerebral cortex, hypothalamus (particularly in neurones of the paraventricular and supraoptic nucleus), hippocampus, thalamus, striatum and pituitary gland. In humans, receptor mRNA is detectable in many peripheral tissues, also consistent with a vascular expression including spleen, thymus, prostate, testis, ovary, intestine. In human brain, mRNA is present in neurones, oligodendrocytes and astrocytes, but was not detected in macrophages or microglia. Apelin receptor-like immunoreactivity is expressed in endothelial cells lining small intramyocardial, renal, pulmonary and adrenal vessels, small coronary arteries, large conduit vessels and in endocardial endothelial cells. Lower levels of receptor are also present in cardiomyocytes and vascular smooth muscle cells of blood vessels from different vascular beds [4].

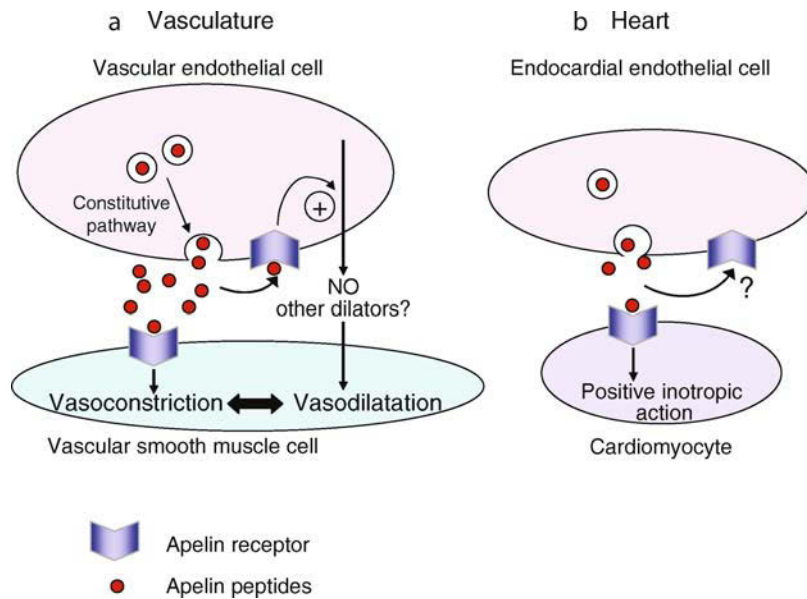
[¹²⁵I]-(Pyr¹)apelin-13 used to characterise native apelin receptors in human heart (atria and ventricles) bound with a single high affinity (▶ K_D = 0.4 nM),

comparable to other vasoactive peptides. ▶Hill slopes were close to unity, with no evidence for a biphasic curve that might indicate further receptor sub-types. Binding was time dependent with an association rate constant (K_{obs}) of 0.115 min⁻¹ and dissociation rate constant of 0.01 min⁻¹. Receptor density in human heart was comparatively low, ~4 fmol mg⁻¹ protein. In rat tissues, [¹²⁵I]-(Pyr¹)apelin-13 bound in the CNS (brain) and periphery (lung) with similar high affinities (K_D = 0.6 nM) [4]. Disruption of the gene encoding the apelin receptor in a *knock-out* mouse abolished, as expected, all apelin responses, supporting the concept that apelins mediate their actions via a single receptor.

Apelin receptors activate several signalling pathways including coupling through inhibitory *G-proteins* (G_i) and Ras-independent activation of extracellular-regulated kinases (ERKs) via protein kinase C (PKC). The apelin receptor is one of number of G-protein-coupled receptors that can act as an alternative co-receptor for entry into cells of HIV and simian immunodeficiency virus (SIV) strains in human U87 cells expressing CD4 in vitro. Apelin peptides blocks entry of HIV but display different potencies, with apelin-36 being more effective than shorter sequences [3].

Role in the Cardiovascular System

In the cardiovascular system, apelins act directly on smooth muscle cells of the vasculature to cause ▶*vasoconstriction*, on apelin receptors on the endothelium to indirectly release ▶*vasodilators* to cause relaxation and on myocyte receptors to increase cardiac contractility (Fig. 3). In animals, short apelin peptides play a role in the regulation of cardiovascular homeostasis. Apelin-13 (~3 µg/kg) transiently lowered ▶*blood pressure* by ~10 mm Hg for 3–4 min in anaesthetised rats in vivo following intravenous injection in a nitric oxide dependent manner. This action was more pronounced in spontaneously hypertensive rats, with apelin-13 (15 µg/kg) lowering blood pressure by about 60%. Concomitant administration of apelin-13(F13A), a synthetic peptide with Ala substituted at residue 13, blocked hypotensive effects of apelin-13 suggesting that apelin-13(F13A) behaved as a functional antagonist [5]. Although apelin receptor knockout mice displayed no significant changes in baseline blood pressure compared to wild type controls, infusion of apelin transiently decreased the blood pressure of wild-type mice, which was abolished in the knock-out. Apelin receptor deficient mice had an increased pressor response to angiotensin II, and the baseline blood pressure of double mutant mice homozygous for both apelin and angiotensin-type 1a receptor was significantly elevated compared with the control. Following removal of the endothelium, [Pyr¹] apelin-13 apelin was a potent vasoconstrictor in human vessels (saphenous vein) in vitro, by a direct action on



Apelins and the Apelin Receptor. Figure 3 Scheme illustrating the hypothesised mechanisms of control of human (a) vascular tone and (b) cardiac contractility by apelin peptides (•). In the vasculature, apelins (released via the small vesicles of the constitutive pathway) may act directly to activate apelin receptors on the underlying smooth muscle to produce vasoconstriction. This response may be modified by apelin peptides feeding back onto apelin receptors on endothelial cells to stimulate the release of dilators, such as nitric oxide. In heart, apelin peptides, released from endocardial endothelial cells, activate apelin receptors on cardiomyocytes to elicit positive inotropic actions.

vascular smooth muscle, suggesting in pathophysiological conditions of endothelial cell dysfunction, vasoconstriction may be the predominant action. A potential role for the apelin receptor system in the pathogenesis of high blood pressure has been proposed

Apelin-16 is a potent positive inotropic agent in rats and in animal models treatment with apelin improves heart function. In isolated perfused rat hearts, infusion of apelin-16 (0.01–10 nM) induced a dose-dependent positive inotropic effect with an EC_{50} value of 33 pM. Continuous infusion of apelin-16 at a rate of 0.01 μ g/min for 20 min significantly increased contractility in rats in heart failure, 6 weeks after left anterior descending coronary artery ligation. These animal studies suggest apelin may have use as an acute inotropic agent in patients with ischemic heart failure. In humans, mRNA microarrays identified the apelin receptor gene as the only one of 12,000 genes tested that showed significant increase in expression levels in heart failure after implantation of a ventricular assist device, suggesting that apelin may play a compensatory role in the early stages of this condition. Apelin mRNA is increased in human myocardium in heart failure and mRNA encoding the receptor is significantly decreased.

Fluid Homeostasis

Apelin receptor and peptides are co-expressed in two nuclei of the hypothalamus, the supraoptic and paraventricular nucleus which play a major role in

the physiological regulation of fluid homeostasis by production of **vasopressin** (ADH). Axonal transport translocates ADH to the posterior pituitary, where it is released in response to osmotic stimuli sensed by hypothalamic neurones in order to regulate water and sodium uptake in the kidney as well as vascular tone. Messenger RNA encoding apelin co-localised in neurones expressing ADH mRNA, suggesting a role in the regulation of fluid homeostasis. Circulating plasma ADH levels decreased (–47%) after intracerebroventricular administration of apelin-13. In mice deprived of water, intracerebroventricular administration of apelin-13 significantly reduced the water intake in the initial 30 min after re-exposure to drinking water, and apelin-17 lowered circulating ADH levels (–43%). Increased water intake was also observed in the first 60 min after intracerebroventricular administration of apelin-13 in rats [3, 4].

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Apical Membrane

Refers to the aspect of epithelial cells facing the mucosal (as opposed to serosal) side of the cells.

► Na^+/H^+ -Exchangers

APJ Receptor

► Apelins

Apolipoproteins

Proteins embedded in the shell of lipoproteins. They serve as scaffold for assembly of the lipoprotein particle in the endoplasmic reticulum. In addition, they control metabolism of lipoproteins in the circulation by interaction with enzymes such as lipases. Finally, apolipoproteins determine cellular uptake of the particles by interaction with specific lipoprotein receptors expressed on the surface of target cells.

- Low-Density Lipoprotein Receptor Gene Family
- Lipoprotein Metabolism Lipid Transfer Protein

Apoptosis

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Synonyms

Programmed cell death

Definition

Apoptosis is the most common form of programmed cell death. Apoptosis is characterized morphologically by cell shrinkage, membrane blebbing (zeiosis), ► **DNA fragmentation**, chromatin condensation, and nuclear fragmentation [1]. Biochemically, these morphological alterations in most cases require the activation of enzymes called caspases. Apoptosis plays a role during normal development where it facilitates the shaping of organs and their plasticity, e.g. in the developing central nervous system [2]. However, apoptosis also takes place in most tissues throughout life. Older or damaged cells undergo apoptosis and they are replaced by new cells which are constantly formed by proliferation of precursor cells. Thus, there is equilibrium between apoptosis and proliferation in the different tissues. A dysbalance in this equilibrium, e.g. by external stress or by a mutation or an epigenetic alteration of an apoptosis pathway constituent which hampers normal execution of the cell death program, is often causative for a disease or a pathological condition. Hence, the interest in understanding the underlying biochemical mechanisms of cell death induction by apoptosis has increased immensely ever since the importance of apoptosis as one part of this equation has been realized.

Basic Mechanisms

Two main apoptosis pathways exist. The first one is the death receptor pathway which can be triggered from the outside of the cell when a so-called death ligand which belongs to the ► **tumor necrosis factor (TNF)** family of cytokines crosslinks its cognate receptor on the surface of a cell. Besides TNF also TRAIL and the CD95 (Fas/APO-1) ligand (CD95L) can directly induce apoptosis and form part of the death ligand subfamily. Cross-linking of a death receptor by its ligand or by an agonistic antibody to the receptor results in the formation of the death-inducing signaling complex (DISC). The formation of this multi-protein complex leads to the activation of the initiator caspases 8 and 10 which both form part of this signaling complex.

The other pathway is triggered when so-called BH3-only proteins interact with other members of the Bcl-2 family on mitochondria. The bcl-2 homology domain 3 (BH3 domain) of the BH3-only proteins binds to other Bcl-2 family members thereby influencing their conformation. This interaction facilitates the release of cytochrome C and other mitochondrial proteins from the intermembrane space of mitochondria. Despite much effort the exact biochemical mechanism which governs this release is not yet fully understood. The release of cytochrome C facilitates the formation of the apoptosome, the second platform for apoptosis initiation besides the DISC. At the apoptosome which is also a multi-protein complex the initiator caspase-9 is activated. At this point the two pathways converge.

Active caspases 8, 9 and 10 can convert caspase-3, the most abundant effector caspase from its pro-form to its active cleaved form. Cleavage of a number of different substrates by caspase-3 and also by caspase-6 and -7 which are two other executioner caspases besides caspase-3 then results in the typical morphology which is characteristic of apoptosis. Yet, the activation of caspase-3 and also of caspase-9 can be counteracted by IAPs, so called inhibitor of apoptosis proteins. However, concomitantly with cytochrome C also other proteins are released from mitochondria, including Smac/DIABLO. Smac/DIABLO and potentially other factors can interact with IAPs and thereby neutralize their caspase-inhibitory activity. This releases the breaks on the cell death program and allows apoptosis to ensue.

There is also crosstalk between the two pathways above the mitochondria. The BH3-only protein BID is cleaved by caspase-8 and -10 which yields truncated BID (tBID), the active pro-apoptotic fragment of BID. Thereby, even in cells in which the direct apoptosis pathway which result from death receptor crosslinking is blocked, e.g. by high expression levels of the x-linked IAP (XIAP), the activity of tBID on mitochondria can result in the activation of caspase-3 because the IAP-imposed block on full caspase-3 activation and caspase-9 activity at the apoptosome is released by Smac/DIABLO.

Pharmacological Intervention Apoptosis Induction

Cancer cells often have a survival advantage over normal cells which is usually established during the transformation process. This is often facilitated by loss of pro-apoptotic factors or the acquisition of anti-apoptotic proteins. One such acquisition led to the identification of ►*Bcl-2*. High expression of anti-apoptotic proteins like *Bcl-2* interferes with release of cytochrome C and Smac/DIABLO by BID or other BH3-only proteins, thus hampering the activation of caspase-9 and caspase-3, thereby interfering with the induction of apoptosis. Intriguingly, however, the cancer cells are often dependent on these changes acquired during transformation. Thus, if it were possible to interfere with the activity of a given factor the transformed cells have become dependent on this would be a very suitable therapeutic target. It now appears that in many types of cancer IAPs and the anti-apoptotic ►*Bcl-2* family members fulfill this criterion. In addition, the triggering of apoptosis from the outside of the cells by TRAIL receptor agonists has shown to be effective in killing tumor cells and to be non-toxic. Importantly, also in combination with conventional chemotherapeutics, novel targeted therapeutics, or radiation therapy these drugs which specifically target TRAIL-R1 (DR4) and/or TRAIL-R2 (DR5) have so far

shown no or only very few dose-limiting toxicities in a number of phase I and II clinical trials. Taken together, there are three main protein families which have been identified in the apoptosis pathways as cancer drug targets, i.e. the apoptosis-inducing TRAIL receptors [3], the anti-apoptotic ►*Bcl-2* family members [4] and the IAPs [5]. As a consequence there are three novel classes of cancer drugs: TRAIL receptor agonists, BH3 mimetics and IAP antagonists. These three classes of novel drugs have recently entered clinical trials and it will be interesting to see how these trials will develop, especially considering combinatorial therapies.

Besides direct apoptosis effectors, there are a number of other drugs which influence the above explained apoptosis pathways more indirectly. This class of drugs includes molecules which inhibit survival pathways like e.g. the Ras/Raf kinase pathway, the NF- κ B pathway and many others. Also inhibitors of survival cytokines which are sometimes produced by cancer cells in an autocrine fashion can render cells susceptible to apoptosis and, hence, effective cancer therapy. These include, but are not limited to, ligands for dependence receptors and cytokines like e.g. interleukin-4.

The combinations of conventional cancer therapeutics with novel targeted drugs, whether they directly or indirectly target the cell's apoptosis pathways will open a plethora of novel intervention strategies for cancer treatment in the future. The results of the preclinical work as well as the first results from clinical trials are very encouraging, perhaps promising. It seems that some of the new combinations which are now possible may finally allow the breaking of tolerance of cancer to most currently used therapies. The results of clinical studies with these new multi-target therapeutic strategies will teach us whether we will have managed to outmaneuver the cancer by depriving it of its capacity to generate a viable therapy-resistant variant.

Apoptosis Prevention

Often cells undergo apoptosis at a stage when they were not yet supposed to die, at least under normal physiological conditions. One such condition is reached when cells are deprived of oxygen. This is the case in stroke and acute myocardial infarction. At the core of the lesion the cells die by ►*necrosis* in both, the ischemic part of the heart and the oxygen-deprived part of the brain, as a consequence of complete deprivation of oxygen for too long a time period. Yet, in the penumbra, i.e. the region surrounding this central necrotic lesion, the cells are only deprived of oxygen for a limited period of time. However, if no intervention takes place, many of the cells in the penumbra die within the next days by apoptosis. Therefore pharmacological intervention has aimed at blocking the death of these cells by interfering with apoptosis. For quite some time caspase inhibitors were thought to be drugs

which are potentially useful in these diseases because of their apoptosis-inhibitory capacity *in vitro*. Currently caspase inhibitors are still being evaluated in the context of acute liver failure and first results are encouraging. However, it was discovered that in order to efficiently block cell death with caspase inhibitors it is necessary to very efficiently block caspase activity (i.e. by more than 98%) at any given time in a cell which has been triggered to undergo apoptosis. Since this is very difficult to achieve *in vivo* the attention has shifted towards inhibitors of the initial triggers of the cascade. Death ligands are one class of such triggers. Inhibition of death ligands can be achieved already outside the cell, i.e. before the apoptosis signal is transmitted to the inside of the cell. Hence, interference with death ligand binding to its cognate receptor(s) should allow for efficient inhibition of apoptosis. Consequently, in cases in which a given death receptor-ligand system has been implicated in a specific pathological conditions it should be possible to intervene therapeutically with a blocker of a given death ligand. In the case of TNF the concept of using TNF blockers, i.e. soluble TNF receptor Fc fusion proteins or anti-TNF antibodies, has been demonstrated to be very efficient in blocking the action of TNF in diseases such as rheumatoid arthritis and psoriasis, amongst many other diseases. Also for CD95L this concept has recently been shown to be very promising: in animal models of e.g. myocardial infarction, stroke, spinal cord injury, acute liver failure and graft-versus-host disease as inhibition of CD95L by CD95 blockers, i.e. soluble CD95 receptor Fc fusion proteins or antibodies to CD95L, has resulted in a therapeutic effect. Consequently, efforts are under way to test whether inhibition of CD95L can interfere with the tissue damage in these different disease situations in humans.

- ▶ Monoamine Oxidases
- ▶ Caspases
- ▶ Matrix Metalloproteinases
- ▶ Cell Cycle Control
- ▶ Insulin Receptor
- ▶ MAP Kinase Cascades
- ▶ Neurodegeneration
- ▶ Cancer, Molecular Mechanism of Therapy

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Apoptotic Executioner Caspases

Apoptotic executioner caspases (caspase-3, -6, -7) constitute a subgroup of the caspase family. These proteases are the workhorses of the apoptotic process as they are responsible for cleaving many down-stream substrates important for cellular morphology, organelle homeostasis, cell cycle arrest, and regulation of transcription and translation.

- ▶ Caspases
- ▶ Apoptosis

Apoptotic Initiator Caspases

Apoptotic initiator caspases (caspase-2, -8, -9 and -10) constitute a subgroup of the caspase family. These caspases are the first to become proteolytically active in the apoptotic cascade. Their activation takes place in multiprotein complexes initiated by pro-apoptotic stimuli, such as TNF α , α -Fas, staurosporine. Once activated, they can process their substrates, which include the apoptotic executioner caspases.

- ▶ Caspases
- ▶ Apoptosis

Appetite

Mood and hedonic value associated with feeding, food intake, foraging, consummatory behaviors, and craving in addiction; complex regulation by food entrainable oscillators in the brain and periphery, neuropeptides (including orexins) and biogenic amines.

- ▶ Orexins
- ▶ Appetite Control
- ▶ Anti-obesity Drugs

Appetite Control

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Synonyms

Control of food intake; Regulation of ingestive behaviour

Definition

Appetite control is a complex function of the brain that regulates feeding behaviour. This function integrates cognitive and emotional factors with a complex array of signals from the gastrointestinal tract and from adipose tissue.

Basic Mechanisms

Feeding behaviour is subjected to both short-term regulation during a single-meal and long-term regulation related to the maintenance of body weight and fat content. As a complex function of the brain, ingestive behaviour is controlled by psychological and cognitive factors such as sociocultural context (e.g. eating habits), experience (sensory preferences) or emotional status (mood). Appetite control also integrates information about the status of peripheral organs, particularly the gastrointestinal tract and adipose tissue [1]. Two main groups of signals can be distinguished: (i) satiety signals secreted from gastrointestinal organs, and (ii) adiposity signals that are proportionate to body fat stores. A key factor of this control system is that energy intake is primarily controlled by adjustment of meal size rather than meal onset, allowing the organism to initiate meals at times that are convenient and to adapt eating patterns to individual constraints (food availability) and activities (circadian rhythm).

Satiety Signals

Satiety-inducing signals are conveyed to the brain by afferent nerve fibers that are sensitive to mechanical or chemical stimulation of the stomach and small intestine during food ingestion. In addition, humoral signals such as ►**cholecystokinin** (CCK) are released upon nutrient stimulation of neuroendocrine cells located in the gastrointestinal wall. These satiety signals converge in the nucleus tractus solitarius in the brainstem and induce meal termination in the absence of hypothalamic control, as demonstrated in decerebrated rats. ►CCK is the paradigmatic humoral satiety signal, and its action has been studied extensively in multiple species

including human. Exogenous administration of ►CCK dose-dependently reduces meal size. This effect is synergistically enhanced by other factors that limit meal size, such as gastric distension. Specific CCK-A receptor antagonists stimulate food intake in rats, indicating that endogenous ►CCK contributes to the termination of meals. However, repeated administration of ►CCK before each meal does not reduce caloric intake of free-feeding mice or rats, because the animals compensate the reduced meal size by increasing the number of meals.

Adiposity Signals

Insulin as a Satiety/Adiposity Signal

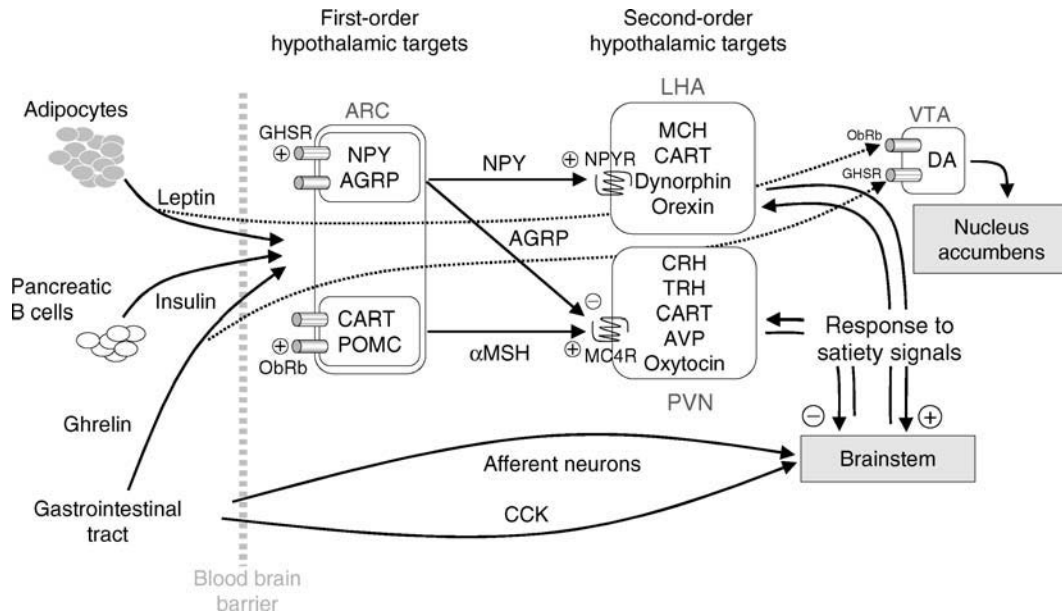
The first hormonal signal found to comply with the characteristics of both a satiety and an adiposity signal was insulin [1]. Insulin levels reflect substrate (carbohydrate) intake and stores, as they rise with blood glucose levels and fall with starvation. In addition, they may reflect the size of adipose stores, because a fatter person secretes more insulin than a lean individual in response to a given increase of blood glucose. This increased insulin secretion in obesity can be explained by the reduced insulin sensitivity of liver, muscle, and adipose tissue. Insulin is known to enter the brain, and direct administration of insulin to the brain reduces food intake. The adipostatic role of insulin is supported by the observation that mutant mice lacking the neuronal insulin receptor (NIRKO mice) develop obesity.

Leptin as an Adiposity Signal

►Leptin is a cytokine produced and secreted by adipose tissue in proportion to the body fat content [3]. Mice and humans lacking leptin or its receptor develop a severe hyperphagia and a dramatic degree of obesity which is considerably more pronounced than that of the NIRKO mouse. Thus, leptin is the key adiposity signal in rodents and humans. Leptin secretion appears to reflect the metabolic status of the adipocyte rather than the sheer size of triglyceride deposits, and leptin levels may transiently be dissociated from total body fat. Nonetheless, over the course of a day with unrestricted food supply, plasma leptin levels reliably reflect the amount of total body fat. Local administration of leptin into the brain results in reduced food intake. The vast majority of patients with obesity have elevated serum levels of leptin. Thus, it is believed that the polygenic obesity is due to leptin resistance rather than to inadequate leptin secretion, or to a reduced blood/brain transport of the cytokine.

Appetite-Regulating Pathways in the Arcuate Nucleus of the Hypothalamus

Two distinct populations of neurons in the arcuate nucleus have been identified as the most relevant target cells of leptin (Fig. 1, [2, 4]). Leptin inhibits expression



Appetite Control. Figure 1 Diagram of pathways integrating appetite control. Satiety is the net output from brainstem centres that leads to the termination of an individual meal. Satiety is primarily determined by neural and humoral inputs from the gastrointestinal tract (satiety signals). Response to satiety signals is modulated by descending anabolic or catabolic pathways originating in the hypothalamus. Appetite regulating hormones are released in proportion to total body fat (leptin), glucose levels (insulin), or changes in food intake (ghrelin). Leptin and insulin stimulate secretion of anorexigenic peptides (α MSH, CART), and inhibit expression of orexigenic ones (NPY, AgRP) in the arcuate nucleus (ARC), whereas ghrelin exerts opposite effects. Secondary target neurons in the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA) integrate signals from neurons in the ARC, and connect them with satiety centers in the brainstem. In addition, recent evidence shows that afferent signals such as leptin and ghrelin also directly target brain centres known to play a key role in reward and addiction. Areas where such neurocircuitry is located include the ventral tegmental area (VTA) or the nucleus accumbens and appear to control mostly the hedonic components of feeding behaviour.

of the orexigenic peptides NPY (▶neuropeptide Y) and AgRP (▶agouti-related protein) in one subset of neurons, and stimulates production of the anorexigenic peptides α MSH (▶ α -melanocyte-stimulating hormone) and ▶CART (cocaine- and amphetamine-regulated transcript) in the other. Insulin receptors are also highly concentrated in the arcuate nucleus, and insulin appears to elicit similar changes in these neuropeptides as leptin.

The Melanocortin Signalling System

Considerable evidence indicates that the molecules of the melanocortin system are key mediators of the response to leptin. AgRP and α MSH are antagonistic ligands for a common receptor, the melanocortin-4 receptor (MC4R). α MSH is an anorexigenic neuropeptide that activates MC4R and thereby reduces appetite, whereas AgRP is an orexigen that acts as an endogenous antagonist of the receptor and suppresses its activation by α MSH. The critical role of the melanocortin system in appetite regulation is supported by the effects of spontaneous and experimental mutations of AgRP, α MSH, and MC4R in mice. Moreover, patients with complete loss of ▶proopiomelanocortin (POMC), the precursor molecule of α MSH, develop severe hyperphagia and overweight,

and 4–5% of all cases of severe human obesity appear to be due to mutations in the MC4R gene.

Role of NPY/AgRP Neurons

NPY has long been known to be a potent orexigen when directly injected into the hypothalamus. Hyperphagia of the leptin-deficient ob/ob mice is attenuated by knockout of NPY, supporting the role of NPY as a downstream effector of leptin. The effects of NPY on appetite regulation appears to be mediated by different receptor subtypes (NPY1R, NPY2R, and NPY5R). However, the neuropeptide is not an indispensable transmitter of adiposity signals, since lean mice which lack NPY show a normal feeding behaviour. On the other hand, it has recently been shown that the NPY/AgRP neurons play an essential role for basal orexigenic drive. If these neurons are completely ablated from the arcuate nucleus, adult mice stop feeding almost completely and loose substantial amounts of body fat. In conclusion, the NPY/AgRP neuron likely plays an essential role in the control of feeding behaviour, but their function can not be easily explained solely based on the expression of the neuropeptides NPY and AgRP.

Second-Order Hypothalamic Targets in Adiposity Signalling

Lesions of the ►lateral hypothalamic area (LHA) cause anorexia, whereas ablation of the ►paraventricular nucleus (PVN) cause a hyperphagic obesity syndrome. Consistent with these results, LHA neurons express the orexigenic neuropeptides MCH and ►orexin. PVN neurons produce several neuropeptides that are anorexigenic when administered directly into the brain (CRH, TRH, oxytocin), in addition to their better known roles as endocrine regulators. LHA and PVN receive rich inputs from axons of NPY/AgRP and α MSH/CART-producing neurons in the arcuate nucleus.

Other Hormones, Peptides, and Neurotransmitters Involved in Appetite Control

Many other peptides including galanin, ghrelin, and ►glucagon-like peptide-1 and 2 (GLP-1/GLP-2) have been described to participate in appetite control (Table 1). In addition, the neurotransmitters norepinephrine, dopamine, and serotonin are known to be involved in appetite regulation. The role of the monoamines in energy homeostasis is illustrated by effects of drugs (see below). Agonists of α_1 adrenoceptors, 5-HT_{2C} serotonin receptors, and dopamine receptors (D1 and/or D2) suppress appetite. However, the relevant neural circuits that use these transmitters are not very well defined. A control system mediating appetite-stimulating effects is the cannabinoid signalling. Recently, endocannabinoids have been added to the list of signals that act downstream of leptin. Leptin reduces levels of the endocannabinoid anandamide in the hypothalamus of normal rats, and mice that lack the cannabinoid receptor 1 (CB1) showed reduced food intake under conditions of low leptin levels (after fasting).

Pharmacological Intervention Appetite-Suppressing Drugs

The increasing prevalence of obesity and its consequences has stimulated the search for appetite-suppressing drugs as anti-obesity agents [5]. Therapy based on nutritional and behavioural counselling produces almost always only a temporary weight loss. The existing drugs that target adrenergic and serotonergic pathways (e.g. metamphetamine, phentermine, fenfluramine, sibutramine) have a negative reputation of toxicity and limited efficacy. The recent insights in appetite control as outlined above have provided new candidate targets for the search of appetite suppressing drugs. Since obesity is usually a chronic disorder which requires life-long therapy, anti-obesity drugs need to meet high safety standards.

β -Phenylethylamine Drugs

The appetite-suppressing effect of β -phenylethylamine drugs is either related to their sympathomimetic effect (metamphetamine, phentermine, diethylpropion), to

increased serotonergic transmission (fenfluramine), or both (sibutramine). Compared with metamphetamine, phentermine and diethylpropion appear to have little abuse potential but exhibit the typical side effects of sympathomimetic drugs (insomnia, hypertension). Use of fenfluramine was terminated after a high incidence of valvular heart disease was reported in patients treated with a combination of phentermine and fenfluramine. The same rationale to combine serotonergic and noradrenergic action underlies the therapy with sibutramine, a serotonin-norepinephrine reuptake inhibitor. Weight reduction by 5–10% was achieved over 24 weeks of treatment with sibutramine in doses from 10–15 mg/d. Weight was regained when the drug was stopped, indicating that a continuous therapy would be necessary to achieve the useful, but limited therapeutic effect. This general limitation is likely to apply for any novel drug that targets central noradrenergic and/or serotonergic pathways, e.g. agonists of the 5-HT_{2C} serotonin receptor.

Cannabinoid-1 Receptor Antagonists

These compounds are a novel class of anti-obesity agents which block the cannabinoid-1 (CB-1) receptor. Rimonabant, the only CB-1 receptor antagonist currently on the market, produces a weight loss of 3.4 or 6.6 kg with daily doses of 5 and 20 mg, respectively. In addition, the agent reduces plasma triglycerides and increases HDL cholesterol. The beneficial effects of rimonabant are probably mediated by both central and peripheral CB-1 receptors. Adverse effects were nausea, diarrhoea, dizziness, anxiety and depression. A second CB-1 receptor antagonist presently being tested in clinical trials is CP 945598.

Incretin-Mimetic Agents

Exenatide, a 39-amino acid peptide from the Gila monster (*Heloderma suspectum*), is a functional analog of human glucagon-like peptide-1 (GLP-1). Because of its resistance to degradation, in-vivo potency of exenatide is much greater than that of GLP-1. Exenatide improves glycemic control through glucose-dependent secretion of insulin, suppression of high glucagon levels in patients with type 2 diabetes, delay of gastric emptying, and reduction of food intake. Exenatide is administered at doses of 5 and 10 μ g twice daily; its most frequent adverse effects were nausea and hypoglycaemia.

Leptin

Leptin has been shown to markedly reduce appetite and weight in the extremely rare individuals who lack leptin. In contrast, in the first clinical study of patients with polygenic obesity and elevated leptin levels, weight loss was variable and relatively small. This disappointing result may be explained by the leptin resistance consistently observed in obese humans and rodents. However, it cannot be excluded that a small

Appetite Control. Table 1 Hormones, peptides and neurotransmitters implicated in appetite control

Hormones, peptides and neurotransmitters	Effect of ICV injection on food intake	Effect of gene deletion on food intake	Response to adiposity signals	Receptor	Effect of receptor defect on food intake
Satiety signals					
cholecystokinin (CCK)	↓		-	CCK-A	↑(↔ ^a)
Adiposity signals					
Leptin	↓	↑↑	—	LEPRb	↑↑
insulin	↓		—	IR	↑ ^b
Orexigenic					
Neuropeptide Y (NPY)	↑	↔(↓ ^{c,d})	↓	NPY2R	↑
				NPY5R ^e	↑
Agouti-related peptide (AgRP)	↑	↔(↓ ^d)	↓	MC4R ^{e,f}	↑
Melanin-concentrating hormone (MCH)	↑	↓	↓	MCHR1	↑
				MCHR2	
Orexin A and B (hypocretins)	↑	↔ ^g	↓	HCRT1	
				HCRT2	
Galanin	↑	↔		GALR1–3	
Ghrelin	↑	↔	↓	GHSR	↓ ^h
Endocannabinoids	↑			CB1	↔(↓ ⁱ)
Anorexigenic					
α-Melanocyte-stimulating hormone (αMSH)	↓	↑ ^j	↑	MC4R	↑
Cocaine- and amphetamine-regulated transcript (CART)	↓	↔	↑	?	
Corticotropin-releasing hormone (CRH)	↓	↔versus ↓	↑	CRHR1	↔(↓ ^k)
				CRHR2	↔
Urocortin	↓		↑	CRHR1	↔s(↓ ^k)
				CRHR2	↔
Thyrotropin-releasing hormone (TRH)	↓		↑	TRHR	
Glucagon-like peptide (GLP-1,2)	↓			GLPR	↔
Serotonin	↓			5-HT _{1B}	↑
				5-HT _{2C}	
Noradrenaline	↓(↑)			α ₁ , α ₂	

^aNormal basal food intake in knockout mice, but stimulatory response to CCK is abolished.

^bNeuron-specific insulin receptor knockout.

^cReduction of hyperphagia was observed in leptin-deficient mice that also lack NPY.

^dNPY/AgRP neuronal ablation.

^eOther receptor isoforms may also be relevant.

^fAgRP acts antagonistic on MC4 receptors.

^gKnockout mice exhibit narcolepsy.

^hMice under chronic high fat diet.

ⁱReduced feeding response to fasting in CB1 knockout mice.

^jαMSH deficiency in patients with mutations in the precursor, proopiomelanocortin (POMC).

^kNormal basal food intake in knockout mice, but inhibitory response to urocortin is attenuated.

subpopulation of obese patients is susceptible to the cytokine. Major efforts are currently underway to develop new drugs that target hypothalamic pathways downstream of leptin (e.g., NPY receptor antagonists, MC4R agonists, CRH agonists).

Drugs with Appetite-Stimulating Effects

Psychotropic Drugs

Stimulation of appetite and weight gain has frequently been observed as a side effect of long-term therapy with various psychoactive drugs. Prominent examples not

only are the tricyclic (e.g. imipramine) and heterocyclic (e.g. mirtazepine) antidepressants but also selective serotonin reuptake inhibitors (e.g. paroxetine), neuroleptic drugs (e.g. olanzapine), and lithium. Although it is reasonable to assume that these drugs interfere with central serotonergic and/or adrenergic signalling, the exact mechanism of their appetite-stimulating effect and the receptors involved are unknown. Stimulation of appetite by cyproheptadine, an antihistamin/antiserotonin agent, is believed to reflect antagonism of serotonin receptors.

Treatment of Cachexia and Anorexia

In the palliative treatment of cachexia and anorexia in advanced cancer and AIDS patients, modest relief can be achieved with appetite-stimulating drugs. Various pharmacologic strategies have been tested, including corticosteroids, anabolic steroids, megestrol acetate, cyproheptadine, melatonin, and dronabinol (delta-9-tetrahydrocannabinol). The cannabinoid receptor agonist dronabinol is approved in the US for stimulation of appetite in AIDS patients. Thalidomide also improves appetite and progressive weight gain in AIDS patients. Megestrol is so far the only agent associated with increased appetite and weight gain in patients with cancer.

- ▶Neuropeptide Y
- ▶Endocannabinoids
- ▶Orexins
- ▶Anti-obesity Drug
- ▶Incretin Hormones

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Aptamers

Oligonucleic acid or peptide molecules that bind a specific target molecule. Nucleic acid aptamer species can be engineered through repeated rounds of in vitro selection to bind to various molecular targets such as

small molecules, proteins, nucleic acids, and even cells, tissues and organisms. Aptamers offer molecular recognition properties, can be engineered completely in a test tube, are readily produced by chemical synthesis, possess desirable storage properties, and elicit little or no immunogenicity in therapeutic applications.

- ▶Tyrosine Kinase Inhibitors
- ▶Antisense Oligonucleotides

aPTT

Activated partial thromboplastin time.

- ▶Anticoagulants

Aquaporins

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Synonyms

Water channels

Definition

Aquaporins (AQP) are a family of integral membrane proteins expressed in all organisms and play a fundamental role in the regulation of water transport into and out of cells.

Basic Mechanisms Background

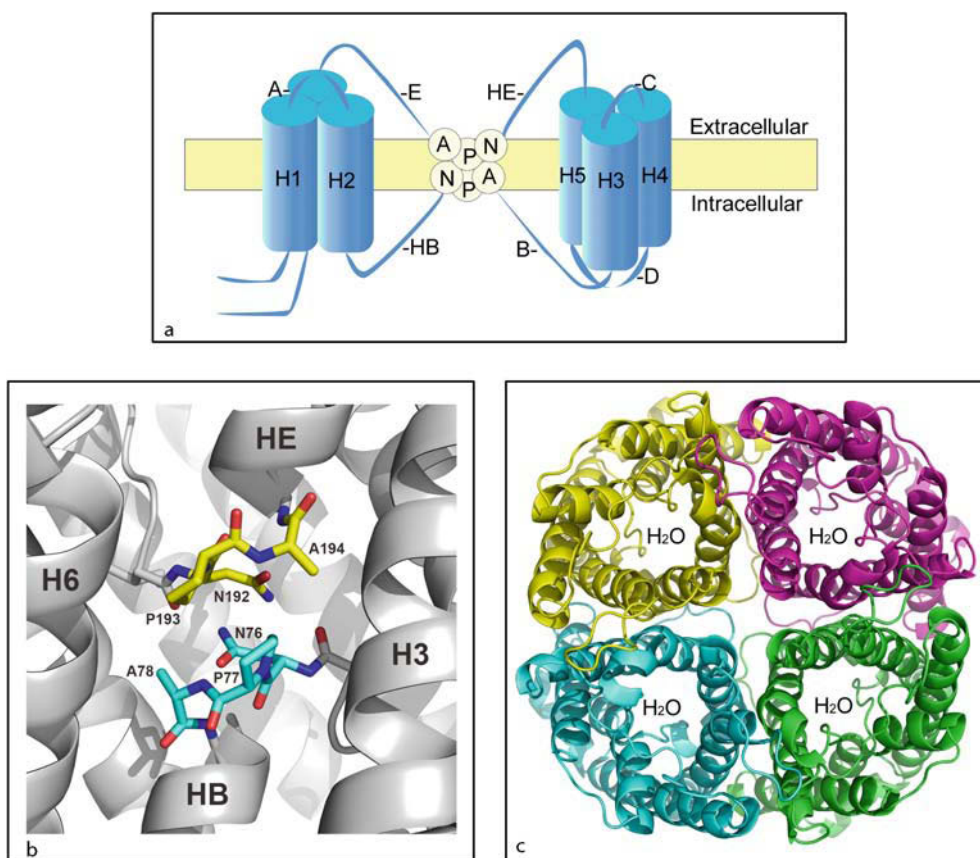
Aquaporins are central players in mammalian physiology, but are also important in microorganisms and plants. The number of AQPs in plants is quite high; angiosperms species, for example, express approximately 35 different AQPs divided into four families on the basis of their sequence. Moreover, plant AQPs might be considered multifunctional channels for their different transport properties.

To date, 13 different AQPs have been identified in mammals (AQP0–AQP12). Most of these proteins allow efficient water transport through the cell membrane. However, several studies revealed that some AQPs also mediate the transport of various small molecules such as hydrogen peroxide, urea, glycerol or anions. On the basis of their different transport properties, AQPs can be divided in two major groups: classical AQPs and aquaglyceroporins. Functional studies revealed that all AQPs transport water to a particular extent. AQP0, 1, 2, 4 and 5 are classical aquaporins being only permeable to water, while AQP3, 7, 9, 10 and 12 can also facilitate the transport of other small molecules, such as glycerol and urea. AQP6 has been suggested to function as a pH-sensitive chloride channel, while AQP8 is permeable to urea and hydrogen peroxide. AQP7 and 9 also transport heavy metal salts, such as arsenite, while AQP9 is additionally permeable to other solutes, such as urea, linear polyols, purines, pyrimidines and nucleosides. The water transport properties of AQP11 are unknown, but it is

impermeable to any of the small molecules mentioned above. Considering the importance of water transport in several biological processes and the expression and distribution of AQPs in all organisms, it's likely that mutations in these genes contribute to various diseases.

Structure of AQPs

Hydrophobicity plots of AQPs indicated that these proteins consist of six transmembrane α -helices (H1–H6 in Fig. 1a) connected by five connecting loops (A–E), and flanked by cytosolic N- and C-termini. The second half of the molecule is an evolutionary duplicate and inverse orientation of the first half of the molecule. Loops B and E of the channel bend into the membrane with an α -helical conformation (HB, HE in Fig. 1b) and meet and each other at their so-called Asn-Pro-Ala (NPA) boxes. These NPA motifs are the hallmark of AQPs and form the actual selective pore of the channel, as at this location, the diameter is of that of a water molecule (3 Å; Fig. 1a and b). Based on the narrowing of the channel from both membrane sides to this small



Aquaporins. Figure 1 (a) The hour-glass model. The scheme depicts the six transmembrane helices (H1–H6), the connecting loops A–E, including the helical parts of loops B ((H)B) and E (E(H)), and the conserved NPA (Asn–Pro–Ala) motif of canonical aquaporins. (b) Structure of the conserved NPA motif region, flanked by the indicated helices. (c) Crystallographic structure of AQP1 tetramer. The four water pores in a tetramer are indicated [1].

pore, an “hour-glass” structure model was proposed, which has later been proven by electron and X-ray crystal studies of AQP1 [1]. Movement of water through the channel occurs in single file and at a speed indifferent from water molecule movement within water. This latter item means that water does not interact with the channel at its narrowest point, i.e., the NPA box region. Indeed, six water molecules form a single file through the channel of AQP1 and hydrogen bonds only occur within the AQP pore between water and six residues (Gly74, Ala75, and His76 on the cytoplasmic side and Gly190, Cys191, and Gly192 on the extracellular side), but not at the NPA motif. At this latter point, water molecules reorient to form a transient hydrogen bond with the conserved asparagine in the NPA triplet, thereby impairing the conduction of protons. Interestingly, the most recently identified AQPs, AQP11 and AQP12, have their E-loop NPA motif, but the alanine in the loop B NPA motif is replaced by cysteine (AQP11) or threonine (AQP12). The functional consequences are at present unclear.

Although freeze-fracture experiments have demonstrated that monomers are assembled into stable tetramers in the membranes, radiation inactivation studies and, later, expression studies revealed that each monomer is a functional water channel (Fig. 1c).

Tissue Distribution of AQPs

The presently known mammalian AQP0-AQP12 have been localized in tissues involved in fluid transport as well as in nonfluid-transporting tissues (Table 1). Most AQPs are constitutively present in the plasma membrane, whereas some water channels can be triggered to shuttle between intracellular vesicles and the plasma membrane [2].

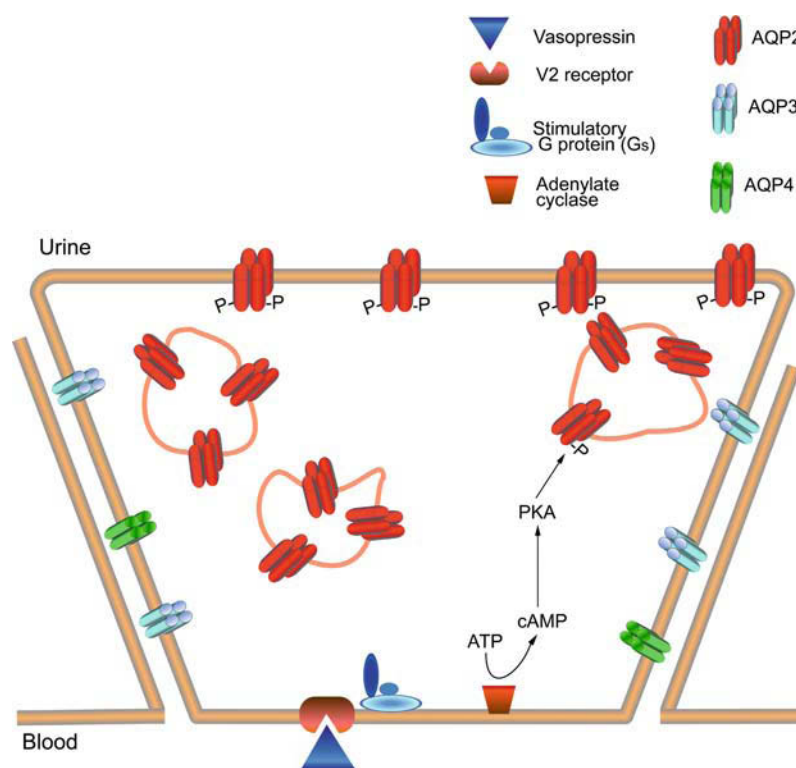
AQP0, formerly known as the Major Intrinsic Protein of 26 kDa (MIP26), is specifically expressed in the plasma membrane of eye lens fiber cells. It transports water to a low degree, but has also been implicated in cell adhesion and gap junction formation. Its main role is to maintain the transparency of the lens by maintaining a tight cellular connection to neighboring cells and/or by controlling the fluid circulation.

AQP1, till the renaming to AQPs referred to as CHIP28, is widely expressed throughout the body. AQP1 is located in the proximal tubule, thin descending limb of Henle and descending vasa recta in the kidney. Outside the kidney, AQP1 is present in endothelial cells of capillaries and small vessels throughout the whole digestive system, including salivary gland, esophagus, stomach, intestine, liver, gallbladder and pancreas. In addition, AQP1 is found in red blood cells, ear, eye, lung, male reproductive system and the choroid plexus. In contrast to its other locations, AQP1 shuttles to the apical membrane of cholangiocytes, which is under hormonal regulation.

The AQP2 water channel is highly expressed in the principal cells of renal collecting duct [3]. Whereas most AQPs are constitutively present on the plasma membrane, AQP2 shuttles between intracellular storage vesicles and the apical membrane. The localization of AQP2 within the principal cell is mainly controlled by the antidiuretic hormone vasopressin. In states of dehydration or hypovolemia, vasopressin is released into blood and binds its receptor on renal principal cells. Binding of vasopressin initiates a cAMP signaling cascade resulting in a fast translocation of AQP2 bearing vesicles to the apical membrane, rendering the membrane permeable to water (Fig. 2). In extrarenal tissues, AQP2 can be found in vas deferens and in the inner ear.

Aquaporins. Table 1 Localization of AQPs

AQP	Localization
0	Eye lens fiber cells
1	Kidney tubules, salivary gland, esophagus, stomach, intestine, liver, gallbladder, pancreas, red blood cells, ear, eye, lung, male reproductive system, the choroid plexus
2	Kidney collecting duct, vas deferens, inner ear
3	Kidney collecting duct, skin, bladder epithelium, digestive tract, respiratory tract, eye, brain
4	CNS, kidney collecting duct, glandular epithelia, airways, skeletal muscle, stomach, retina and ear
5	Glandular epithelia, lung epithelium, gastrointestinal tract, pancreas, ear
6	Kidney collecting duct
7	Adipose tissue, kidney proximal tubule, testis, gastrointestinal tract, immature dendritic cells and ear
8	Gallbladder, liver, pancreas, intestine, salivary gland, testis, heart, kidney, lung, placenta
9	Liver, intestinal wall, lung, leukocytes, testis, ear, brain
10	Small intestine
11	Kidney, liver, testis, brain
12	Pancreatic acinar cells



Aquaporins. Figure 2 Model showing the vasopressin dependent AQP2 trafficking in renal collecting duct cells. Vasopressin binding to its V2 receptor results in the activation and dissociation of a stimulatory G protein (Gs), of which the GTP-bound α -subunit activates adenylyl cyclase. The consequent increase in cAMP activates protein kinase A, which phosphorylate several proteins, including AQP2 at Ser256, which is located in its C-terminus. This event is essential for the steady-state redistribution of AQP2 to the apical membrane. Upon restored water homeostasis, blood AVP levels are decreased and AQP2 will be internalized from the apical membrane.

Similar to AQP1, AQP3 expression can be found in many different tissues, including kidney collecting duct, bladder epithelium, digestive tract, respiratory tract, eye and brain. In kidney, AQP3 is present in the basolateral membrane of collecting duct principal cells. In the digestive tract, AQP3 is expressed in epithelial cells ranging from the oral cavity to the stomach and from the distal colon to the anus. AQP3 is also expressed in skin, where it is involved in skin hydration and elasticity.

AQP4 is the predominant water channel in the central nervous system (CNS), where it is involved in maintaining brain water balance and neural signal transduction. It is mainly expressed in astroglial cells, which support the neurons. Outside the CNS, AQP4 has been found in the basolateral membrane of renal principal cells as well as in various glandular epithelia, airways, skeletal muscle, stomach, retina and ear.

AQP5 is expressed in lung epithelium, gastrointestinal tract, pancreas and ear. Several glandular epithelia also express AQP5, including airway submucosal glands, salivary glands, lachrymal glands and sweat glands suggesting a role for AQP5 in the release of airway fluids, saliva, tears and sweat.

AQP6 is expressed in the intercalated cells of the kidney collecting duct. This channel is hardly permeable to water, but capable of transporting anions, including chloride, and is therefore thought to play a role in maintenance of body acid-base balance or in intracellular vesicle acidification.

AQP7 is expressed in the proximal tubule of the kidney, testis, gastrointestinal tract, immature dendritic cells and ear. This glycerol channel is also highly expressed in adipocytes where it is thought to control the release of triglycerides.

Also AQP8 is widely expressed in organs including liver, pancreas, intestine, salivary gland, testis, heart, kidney, lung and placenta. AQP8 expression in gallbladder epithelium suggests a role in secretion of bile. Several groups also found AQP8 to be expressed in mitochondria, but a role for AQP8 in mitochondrial processes remains to be established.

AQP9, a channel highly permeable to water and solutes, is localized in liver, intestinal wall, lung, leukocytes, testis, ear and brain. Liver AQP9 is suggested to act in conjunction with AQP7 in fat metabolism. While AQP7 is involved in the release of glycerol from adipocytes, AQP9 facilitates its transport

into the hepatocytes. Based on its putative function in hepatocytes, AQP9 expression in the brain is suggested to play a role in brain energy metabolism.

AQP10 has only been identified in the small intestine so far and is thought to play a role in hormonal secretion. AQP11 is expressed in kidney, liver, testis and brain, but no function has been found so far. AQP12 has been identified in pancreatic acinar cells, where it is thought to facilitate the release of digestive enzymes into the pancreatic duct.

AQP-associated Pathologies

Knocking out genes and identification of mutations in the human genes provide information on the role of AQPs in normal physiology. The lack of some AQPs directly results in a disease phenotype, while the physiological role of many becomes clear when the putative function is challenged.

Mutations in two genes directly lead to a disease. Mutations in the AQP0 gene lead to dominantly inherited cataract. Single amino acid substitution in the AQP0 gene in both mice and humans result in proteins with impaired trafficking to the plasma membrane and cataract formation, due to loss of the integrity of the lens.

Mutations in the AQP2 gene can cause severe problems, as they result in nephrogenic diabetes insipidus (NDI), a disorder in which patients are unable to concentrate their urine. Congenital NDI can be caused by mutations in the V2R gene (X-linked NDI) or the AQP2 gene (autosomal recessive or dominant NDI) [4, 5]. Missense mutations in the AQP2 gene in recessive NDI affect amino acids located in between the first and last transmembrane domain. Although they might be functional water channels, the mutation leads to a misfolded protein, which is retained by the cellular quality control of the endoplasmic reticulum. In dominant NDI, the mutations are found in the AQP2 C-terminus, resulting in AQP2 mutants that are, in complex with wild-type AQP2, sorted to other subcellular destinations than the apical membrane. Consequently, inadequate amounts of AQP2 are expressed in the apical membrane, resulting in reduced water absorption and increased urine volumes.

Humans lacking AQP1 do not suffer from any severe symptoms. The only phenotype that can be observed in these individuals is a mild renal concentration defect.

Still, the list of diseases in which AQPs may play a role is expanding. AQP3 knockout mice developed NDI and showed polyuria. In addition, these mice showed dry skin and delayed wound healing. Humans lacking a functional AQP3, however, are symptom less. AQP4 knockout mice revealed an important role for AQP4 in recovery after brain injury. AQP5 expression is decreased in patients with Sjögren's syndrome, a disorder characterized by dry eyes, dry mouth and pulmonary problems, but a causal relationship has not

been established yet. AQP7 expression is reduced in obese people compared to lean people, which may indicate that a lack of AQP7 in adipocytes may underlie congenital forms of obesity. The lack of AQP11 results in polycystic kidneys in mice, but whether such a relationship exists in humans is unknown.

Pharmacological Interventions

Although aquaporins play a fundamental role in the regulation of water homeostasis, specific pharmacological therapies are still not available. Several substances like mercurial derivatives, silver, and gold have been demonstrated to inhibit water permeability mediated by aquaporins in oocytes, but most of them are nonspecific and too toxic to be used *in vivo*. Recent studies have shown that tetra-ethyl ammonium (TEA) selectively inhibits AQP1, AQP2 and AQP4 but not AQP3 and AQP5, indicating that quaternary ammonium compounds and in particular TEA may be a good lead compound for the development of specific aquaporin inhibitors.

► Vasopressin/Oxytocin

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Aquaretic Agents

Substances which promote the elimination of water by the kidney without major losses of salts (e.g. conivaptan, tolvaptan, SR121463A/B). They are particularly useful in situations where excess water needs to be eliminated without affecting the salt metabolism, like eu- or hypervolemic hyponatraemia, congestive heart failure, some stages of hypertension and some metabolic states.

► Vasopressin/Oxytocin

► Aquaporins

Arachidonic Acid

The 20 carbon fatty acid, 5,8,11,14 – eicosatetraenoic acid, an essential fatty acid that serves as a precursor for prostaglandins.

► Prostanoids

Area Postrema

The area postrema is a circumventricular brain region positioned on the dorsal surface of the medulla on the floor of the fourth ventricle. The blood–brain barrier and the cerebrospinal fluid–brain barrier are absent in this region and consequently many substances that do not pass across capillaries in other regions of the brain can do so in the area postrema. The chemoreceptor trigger zone (CTZ), located in the lateral area postrema is sensitive to blood-borne emetogens. Nerves from the CTZ connect with the vomiting centre.

► Emesis

Area under the Curve

Area under the Curve (AUC) refers to the area under the curve in a plasma concentration-time curve. It is directly proportional to the amount of drug which has appeared in the blood (“central compartment”), irrespective of the route of administration and the rate at which the drug enters. The ► [bioavailability](#) of an orally administered drug can be determined by comparing the AUCs following oral and intravenous administration.

► Pharmacokinetics

L-Arginine

A substrate for the synthesis of NO that has a potential for improving endothelial dysfunction.

► Nitric Oxide (NO)
► NO Synthases

Arginine Vasopressin

► Vasopressin/Oxytocin

Aromatase

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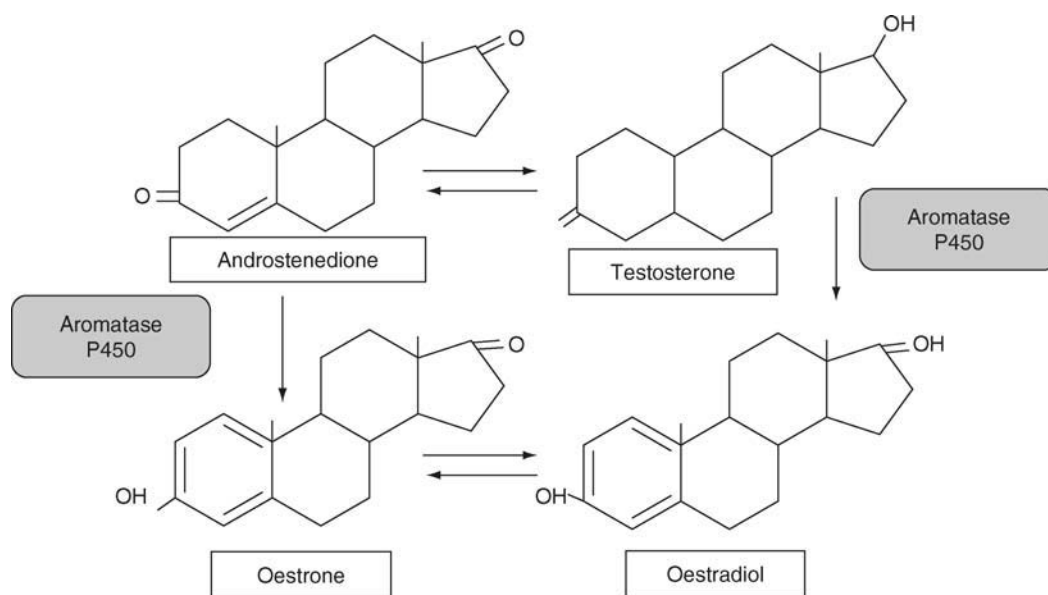
Definition

Aromatase is a cytochrome P450, hemoprotein-containing enzyme, located in the endoplasmic reticulum, which catalyzes the rate-limiting step in the conversion of ► [androgens](#) (androstenedione and testosterone), to ► [estrogens](#) (estrone and estradiol). Agents that inhibit aromatase (aromatase inhibitors) are widely used to prevent the development and progression of ► [estrogen](#) dependent breast cancers.

Basic Characteristics

Estrogen biosynthesis is mediated by the aromatase enzyme, which is a product of the CYP19 gene. The aromatase enzyme is a complex that consists of cytochrome P450 hemoprotein and a flavoprotein, NADPH-cytochrome P450 reductase. This complex is responsible for catalyzing the conversion of steroidal C-19 androgens (androstenedione and testosterone) to C-18 estrogens (estrone and estradiol), which is the rate-limiting final step in the synthesis of estrogens ([Fig. 1](#)). This enzymatic reaction is comprised of three steps each of which requires 1M equiv. of NADPH and oxygen. The first step involves hydroxylation of the androgen substrate at C-19 to produce a 19-hydroxy intermediate. In the second step the 19-hydroxy intermediate is oxidized to produce a 19-oxo compound. The last step in the aromatization reaction is less well defined but is thought to involve the oxidative cleavage of the C10-19 bond to produce ► [estrogens](#) (estrone and estradiol) and formic acid.

Aromatase activity, and hence the capacity to synthesize estrogens, is found in a variety of tissues in the body. Gonadal sites include the ovaries in premenopausal women and the testes in men. Important extragonadal sites of aromatase activity include the placenta, chondrocytes and osteoblasts of bone, adipose tissue, muscle and brain. Aromatase plays an important



Aromatase. Figure 1 Androgens converted to estrogens by aromatase.

role in a number of important biological processes including breast development during puberty and uterine growth and bone maturation during adolescence. In adults aromatase influences bone mineralization, lipid metabolism and cardiovascular risk. In pregnant women it protects against the virilizing effects of fetal androgens.

Drugs

Estrogen is known to be an important stimulus in the development and progression of some breast tumors. Thus targeting the disruption of either the synthesis (i.e., inhibiting aromatase enzyme) or the activity (i.e., blocking [estrogen receptors](#)) of estrogens are potential mechanisms for the prevention and treatment of hormone sensitive breast cancer. Since aromatization is a unique reaction and is the terminal step of the estrogen biosynthetic pathway, agents that block this reaction would not potentially affect the production of other steroids.

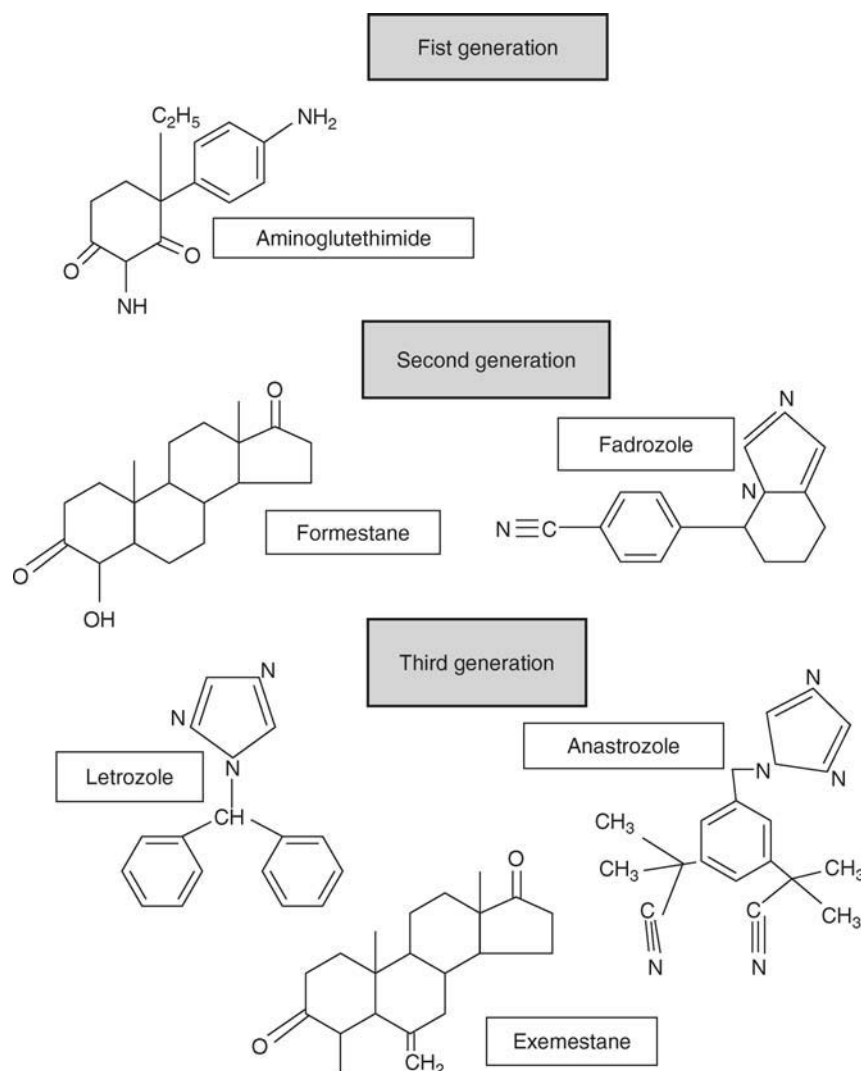
In premenopausal women the ovary is the richest source of aromatase and hence estrogen. Aromatase is confined to the granulosa cells and is produced under the influence of [gonadotropins](#) (FSH and LH). Despite being a rich source of aromatase, three separate studies have shown that aromatase inhibitors are unable to sufficiently suppress ovarian estrogen production to postmenopausal levels. One explanation for this phenomenon may be a compensatory rise in gonadotrophins which maintains adequate estrogen production, despite the presence of the inhibitor. As such aromatase inhibitors cannot be used in premenopausal breast cancer patients. After menopause, ovarian

production of estrogen ceases. However estrogen production continues from peripheral sources of aromatase activity that convert adrenal androgens to estrogens. Aromatase inhibitors have been shown to adequately suppress estrogen production in postmenopausal women, and in this setting are used in the treatment of both early and advanced stage [estrogen receptor](#) positive breast cancer.

Over the last 30 years a number of aromatase inhibitors ([Fig. 2](#)) have been developed. The first (amino-glutethimide) and second (Fadrozole and Fromestane) generation aromatase inhibitors are not commonly used due to their lack of specificity in inhibiting the aromatase enzyme and associated significant side-effects. Two types of third generation aromatase inhibitors are commercially available and have been shown to be either equal to or superior to tamoxifen in the treatment of metastatic estrogen receptor positive breast cancer. Type I (suicidal, noncompetitive) inhibitors bind irreversible with the aromatase enzyme thereby permanently blocking its activity. Exemestane is an example of a type I inhibitor. Type II inhibitors bind reversible with the aromatase enzyme, examples of which include letrozole and anastrozole.

Aminoglutethimide

Aminooglutethimide was the first aromatase inhibitor to be used in patients with metastatic breast cancer, where response rates of up to 30% have been reported. Unfortunately, due to its lack of selectivity for aromatase, it induced a medical adrenalectomy that resulted in suppression of aldosterone and cortisol. With the development of more selective aromatase



Aromatase. Figure 2 Aromatase Inhibitors.

inhibitors, aminoglutethimide is now rarely used for the treatment of breast cancer. It is occasionally used for the treatment of medical conditions involving excess hormone production such as [Cushing's syndrome](#).

Anastrozole

Anastrozole is a nonsteroidal, type II, aromatase inhibitor that is 200 times more potent than aminoglutethimide. It is eliminated primarily via hepatic metabolism, has a terminal half life of 50 h with steady state concentrations achieved approximately 10 days with once daily dosing regimens. It is administered orally at a dose of 1 mg/day that achieves near maximal aromatase inhibition and hence estrogen suppression in breast cancer patients. No effect on adrenal steroidogenesis has been observed at up to ten times the daily recommended dose. When used in the metastatic setting, anastrozole has been shown

to increase time to progression when compared to tamoxifen. In the ATAC (Arimidex, [Tamoxifen](#), Alone or in Combination) trial over 9,000 patients with early stage breast cancer were randomized to 5 years of anastrozole 1 mg/day or 5 years of tamoxifen 20 mg/day or a combination of both. At a median follow up of 33 months, 47 months, and 68 months, compared to tamoxifen, anastrozole upfront significantly increased disease free survival and time to recurrence and reduced the risk of contra lateral breast cancer. The combination arm of the trial was closed as it was no more efficacious than tamoxifen alone. Currently trials are ongoing evaluating anastrozole as chemopreventive agent for breast cancer.

Letrozole

Like anastrozole, letrozole is a third generation, type II nonsteroidal aromatase inhibitor. Renal excretion of its

inactive glucuronide metabolite represents its main pathway of clearance. It has a half-life of 2 days and at the recommended daily dose of 2.5 mg steady-state plasma levels is reached in 2–6 weeks. Letrozole has proved effective when used either sequentially after tamoxifen or upfront in the treatment of patients with early stage breast cancer. The MA-17 trial randomized approximately 5,000 postmenopausal breast cancer patients who had received 5 years of tamoxifen to either placebo or 5 years of letrozole. The Breast International Group (BIG) 1–98 trial randomized postmenopausal breast cancer patients to 5 years of tamoxifen, 5 years of letrozole, or 2 years of either agent (i.e., tamoxifen or letrozole) followed by three years of the other agent (i.e., tamoxifen or letrozole). In terms of disease free survival the MA-17 trial showed an advantage to switching to letrozole and the BIG 1–98 trial showed an advantage to up front letrozole with results awaited for the switching group.

Exemestane

Exemestane is a type II, steroidal aromatase inhibitor with an androgen structure. It is metabolized by CYP3A4 enzyme and has a half-life of 27 h. At the recommended once daily dose of 25 mg no effect is seen on adrenal steroid production and maximal estrogen suppression is achieved in 7 days. In early breast cancer treatment it has been studied as a sequential agent after several years of tamoxifen. The Intergroup Exemestane Study (IES) randomized over 4000 patients to either 5 years of tamoxifen 20 mg/day or 2–3 years of tamoxifen followed by exemestane 25 mg/day. A significant reduction in disease free survival (hazard ratio, 0.76, $p = 0.0001$) and risk of contralateral breast cancer favoring the group switching to exemestane was observed.

Side Effects

Aromatase inhibitors are relatively well tolerated; however have a number of distinct side effects are observed that stem from the state of estrogen deprivation induced by aromatase inhibitors. Side effects include hot flashes, joint and muscle aches, vasomotor symptoms and vaginal dryness. Variable effects of aromatase inhibitors on lipid levels have been observed. Trials comparing third generation aromatase inhibitors to tamoxifen have also reported an increased risk of cardiovascular events in the group receiving aromatase inhibitors.

Other Uses

Aromatase inhibitors have also been used in premenopausal women for the treatment of ►endometriosis and to induce ovarian folliculogenesis as part of the treatment for infertility.

►Sex Steroid Receptors

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Aromatase Inhibitor

An aromatase inhibitor is a class of antiestrogens that inhibits the enzyme aromatase and by that means lowers the level of the estrogen estradiol. Aromatase catalyzes the conversion of testosterone to estradiol in many tissues including the adrenal glands, ovaries, placenta, testicles, adipose tissue, and brain. Estrogen is produced directly by the ovaries and is also made by the body using aromatase. Aromatase inhibitors cannot do anything about estrogen produced by the ovaries, but they do interfere with the body's use of aromatase.

►Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

►Aromatase

►Targeted Cancer Therapy

►P450 Mono-Oxygenase System

Arousal

Arousal is a state of vigilance regulated by subcortical parts of the nervous system, especially connections between the nuclei of the amygdala, the hypothalamus and the brain stem. These unconscious responses prepare the body for action.

In terms of sleep/wake regulation, the arousal systems are those that have highest activity during wake, for example the aminergic (noradrenaline, 5-HT, histamine) systems. The arousal systems inhibit, and are themselves inhibited by the GABAergic system emanating from the ventrolateral preoptic nucleus (VLPO), in a so-called “flip flop” arrangement that is stabilised via orexinergic activity.

►Psychostimulants

►Sleep

Array

Refers to the physical substrate to which biological samples are attached to create features (spots). In gene expression profiling arrays are hybridized with labeled sample and then scanned and analyzed to generate data.

- Microarray Technology

Arrestins

Arrestins act as adaptor proteins that bind to phosphorylated G protein-coupled receptors (GPCR) and link the receptors to clathrin-coated pits. β -Arrestins are essential in the internalization of many GPCRs.

- β -Adrenergic System
- G-protein-coupled receptors
- Tolerance and Desensitization

Arrhythmias

- Antiarrhythmic Drugs

Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

Synonyms

ARVD/C

Definition

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is an inherited heart disorder with progressive replacement of right ventricular muscle by adipose and fibrous tissue. ARVD/C is associated with arrhythmia of right ventricular origin that may result in sudden death. Nine different loci are currently associated with ARVD/C, and causative mutations have so far been identified in five genes, RyR2, TGF β -3, plakoglobin, desmoplakin and plakophilin.

- Ryanodine Receptor

Arteriogenesis

Arteriogenesis is the growth of collateral vessels from a pre-existing arteriolar network to bypass an ischemic area (e.g., following cardiac ischemia).

- Angiogenesis and Vascular Morphogenesis

Arteriosclerosis

- Atherosclerosis

Arylhydrocarbon Receptor

Synonyms

AhR

Definition

Members of the CYP1 family and some other drug metabolizing enzymes including UGT1A6 are collectively induced by polycyclic aromatic hydrocarbons (PAH) and other ligands (e.g. 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) of this basic helix-loop-helix (bHLH) transcription factor. In the absence of ligand AhR is inactive while bound to HSP90 in the cytoplasm. Upon binding of ligand, it moves to the nucleus and dimerizes with Arnt (AhR nuclear translocator protein) to form the active transcription factor that binds to xenobiotic response elements (XRE) in the promotor regions of target genes. AhR furthermore plays a role in regulating hepatic cell regeneration.

- P450 Mono-Oxygenase System
- P450 Enzymes
- Nuclear Receptor Regulation of Drug-Metabolizing
- Dioxins
- PAS Domain

L-Ascorbic Acid

- Vitamin C

ASF Family of Transporters

The **Amphiphilic Solute Facilitator** family of transporters are simple in the sense that no specific source of energy is used for operation (such as hydrolysis of ATP or gradients of inorganic solutes).

- Organic Cation Transporters

Aspirin

Aspirin is the brand name of acetylsalicylic acid. It is the most widely used analgesic, antipyretic and anti-inflammatory drug. Its main mode of action is irreversible acetylation of cyclooxygenases.

- Cyclooxygenases
- Non-steroidal Anti-inflammatory Drugs

Asn-linked Glycosylation

Asn-linked glycosylation is the addition of carbohydrate groups to peptides or proteins through specific glycosyltransferases. Glycosyltransferases within the lumen of the endoplasmic reticulum recognize an Asn-X-Ser/Thr motif (X can be any amino acid but not proline) and link carbohydrates via N-acetylglucosamine to the amino group of the asparagine residue.

- Protein Trafficking and Quality Control
- Intracellular Transport
- Palmitoylation
- Endothelins

Aspirin-like Drugs, Inflammation

- Non-steroidal Anti-inflammatory Drugs

Asthma

- Bronchial Asthma

ASON

ASON stands for antisense oligonucleotides.

- Antisense Oligonucleotides

Astrocytes

Category of glial cells in the vertebrate central nervous system with long radial processes. Astrocytes provide structural support to nerve cells and help to control their chemical and ionic extracellular environment.

- Interferons

Aspartyl Proteinases

Aspartyl proteinases are proteinases that utilize the terminal carboxyl moiety of the side chain of aspartic acid to effect peptide bond hydrolysis.

- Non-viral Peptidases

Atherogenesis

Definition

Atherogenesis is the process that leads to changes in the arterial blood vessels, including deposition of cholesterol (atherosclerosis). It is the pathophysiological process behind the vast majority of heart attacks.

- Atherosclerosis
- HMG-CoA-Reductase Inhibitors (Statins)

Atherosclerosis

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Synonyms

Arteriosclerosis (see definition)

Definition

Atherosclerosis, from the Greek words *athera* – porridge, and *sclereni* – hardening, literally is a hardening of medium- and large-sized arteries specifically due to an atheromatous plaque, whereas arteriosclerosis is a more general term describing any hardening of medium and large arteries. A typical atherosclerotic **▶plaque** consists of a fibrous cap (A structure composed of a dense collagen-rich extracellular matrix with occasional smooth muscle cells, macrophages and T-cells that typically overlies the characteristic central lipid core of plaques.) overlying a lipid-rich core. In addition to the long recognized lipid accumulation, these lesions, known as atheromata, also harbor inflammation and cell recruitment and turnover (proliferation and death). Collectively, the process of atheroma development within an individual is called atherogenesis, and the overall result of the disease process is termed atherosclerosis.

Basic Mechanisms

General

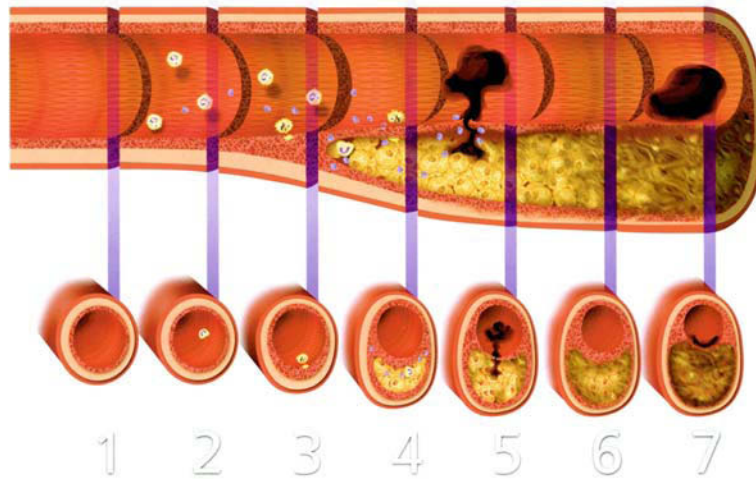
The traditional view of atherosclerosis as a bland cholesterol storage disease falters in the wake of extensive evidence that inflammation plays a central role in all stages of this pathology, from the initial lesion to the final devastating thrombotic complications [1, 2]. Atherosclerosis is a chronic systemic disease preferentially affecting particular circulatory beds; in the coronary arteries, it commonly causes **▶angina pectoris** and **▶myocardial infarction**, in the central nervous system it often leads to transient ischemic attack and **▶ischemic stroke**, and in the periphery it results in **▶intermittent claudication** and critical limb ischemia. The renal and splanchnic beds can also develop atherosclerosis. This disease may manifest clinically with chronic symptoms, such as stable angina or intermittent claudication, acutely as in myocardial infarction or cerebrovascular accident, or may remain clinically silent. Despite a broad array of pharmacologic and procedural interventions to combat this scourge, atherosclerosis remains the leading cause of death and disability in the developed world.

Lesion Initiation and Development of the Fatty Streak

Inflammation participates in atherosclerosis from its inception and onwards (Fig. 1). Even children can develop the “fatty streak,” the initial lesion of atherosclerosis. Fatty streaks do not cause symptoms, and may progress to more complex lesions, or eventually disappear. Fatty streaks have focal increases in the content of lipoproteins within regions of the **▶intima** where they associate with constituents of the extracellular matrix such as proteoglycans, slowing their egress. This retention sequesters lipoproteins within the intima, isolating them from plasma antioxidants, thus favoring their oxidative modification. Oxidatively modified low-density lipoprotein particles (LDL) comprise an incompletely defined mixture, as both the lipid and protein moieties can undergo oxidative modification. Constituents of such modified lipoprotein particles can induce a local inflammatory response.

Endothelial cells (ECs) normally resist leukocyte adhesion. Proinflammatory stimuli that include high-saturated-fat diet, hypercholesterolemia, obesity, hyperglycemia, insulin resistance, hypertension, and smoking trigger the endothelial expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and P-selectin that mediate the attachment of circulating monocytes and lymphocytes. Interestingly, atherosclerotic lesions often form at bifurcations of arteries, regions characterized by a disturbed blood flow which reduces the activity of endothelial atheroprotective molecules. Laminar flow induces the expression of atheroprotective genes such as the antioxidant enzyme superoxide dismutase as well as the synthase that produces nitric oxide, an endogenous vasodilator that can also limit inflammation, for example by limiting VCAM-1 expression.

Chemoattractant factors that include monocyte chemoattractant protein-1 (MCP-1) produced by vascular wall cells in response to modified lipoproteins, direct the migration and diapedesis of adherent monocytes. MCP-1 binds to CCR2 (a chemokine receptor containing two adjacent cysteine residues) on the surface of the migrating monocyte to exert this effect. Experimental evidence and human observations support the involvement of several other chemokines in leukocyte recruitment into the nascent atherosclerotic lesion, including IL-8 and fractalkine. Within the intima, monocytes mature into macrophages under the influence of macrophage colony-stimulating factor (M-CSF), overexpressed in the inflamed intima. M-CSF stimulation also leads to increased macrophage expression of **▶scavenger receptors**, members of the pattern-recognition receptor superfamily, which engulf modified lipoproteins through receptor-mediated endocytosis. Accumulation of cholesterol esters in the cytoplasm changes macrophages into **▶foam cells**, i.e. lipid-laden macrophages characteristic



Atherosclerosis. Figure 1 Initiation, progression, and complication of human coronary atherosclerotic plaque.

Top, Longitudinal section of artery depicting “timeline” of human atherogenesis from normal artery (1) to atheroma that caused clinical manifestations by thrombosis or stenosis (5–7). Bottom, Cross sections of artery during various stages of atheroma evolution. 1, Normal artery. Note that in human arteries, the intimal layer is much better developed than in most other species. The intima of human arteries contains resident smooth muscle cells (SMCs) often as early as first year of life. 2, Lesion initiation occurs when endothelial cells, activated by risk factors such as hyperlipoproteinemia, express adhesion and chemoattractant molecules that recruit inflammatory leukocytes such as monocytes and T lymphocytes. Extracellular lipid begins to accumulate in intima at this stage. 3, Evolution to fibrofatty stage. Monocytes recruited to artery wall become macrophages and express scavenger receptors that bind modified lipoproteins. Macrophages become lipid-laden foam cells by engulfing modified lipoproteins. Leukocytes and resident vascular wall cells can secrete inflammatory cytokines and growth factors that amplify leukocyte recruitment and cause smooth muscle cell migration and proliferation. 4, As lesion progresses, inflammatory mediators cause expression of tissue factor, a potent procoagulant, and of matrix-degrading proteinases that weaken fibrous cap of plaque. 5, If fibrous cap ruptures at point of weakening, coagulation factors in blood can gain access to thrombogenic, tissue factor-containing lipid core, causing thrombosis on nonocclusive atherosclerotic plaque. If balance between prothrombotic and fibrinolytic mechanisms prevailing at that particular region and at that particular time is unfavorable, occlusive thrombus causing acute coronary syndromes may result. 6, When thrombus resorbs, products associated with thrombosis such as thrombin and mediators released from degranulating platelets, including platelet-derived growth factor (PDGF) and transforming growth factor- β (TGF- β), can cause healing response, leading to increased collagen accumulation and smooth muscle cell growth. In this manner, the fibrofatty lesion can evolve into advanced fibrous and often calcified plaque, one that may cause significant stenosis, and produce symptoms of stable angina pectoris. 7, In some cases, occlusive thrombi arise not from fracture of fibrous cap but from superficial erosion of endothelial layer. Resulting mural thrombus, again dependent on local prothrombotic and fibrinolytic balance, can cause acute myocardial infarction. Superficial erosions often complicate advanced and stenotic lesions, as shown here. However, superficial erosions do not necessarily occur after fibrous cap rupture, as depicted in this idealized diagram. (Libby P (2001) Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation* 104:365–372.)

of the early stages of atherosclerosis. In parallel, macrophages proliferate and amplify the inflammatory response through the secretion of numerous growth factors and cytokines, including tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β).

T-cells, representing the adaptive arm of the immune response, also play a critical role in atherogenesis, and enter lesions in response to the chemokines inducible protein-10 (IP-10), monokine induced by IFN- γ (MIG), and IFN-inducible T-cell α -chemoattractant (I-TAC), which bind CXCR3 (a chemokine receptor containing two cysteine residues separated by one amino acid), highly expressed by T lymphocytes in the plaque. The

CD4 $^{+}$ subtype, which recognizes antigens presented as fragments bound to major histocompatibility complex (MHC) class II molecules, predominates in the lesion. Interestingly, CD4 $^{+}$ T-cells reactive to the disease-related antigens associated with oxidized LDL have been cloned from human lesions. The atherosclerotic lesion contains cytokines that promote a Th1 response, inducing activated T-cells to differentiate into Th1 effector cells. These cells amplify the local inflammatory activity by releasing proinflammatory cytokines such as IFN- γ and CD40 ligand (CD40L, CD154).

The atherosclerotic plaque overexpresses another chemoattractant, eotaxin, that may mediate mast cell

migration to the lesion through CXCR3. Multiple studies suggest that mast cells promote atherogenesis by releasing cytokines such as TNF- α , IFN- γ , and IL-6 as well as chemokines that attract other leukocytes and proteases that activate matrix metalloproteinases (MMPs) and participate in arterial remodeling. Although the subject of much debate for several decades, recent results in mice support a role for this cell type in lesion progression and complication.

Progression to Complex Plaque

Macrophages, T-cells, and mast cells infiltrate the lesion and localize particularly in the shoulder region where the atheroma grows. While accumulation of foam cells characterizes fatty streaks, deposition of fibrous tissue defines the more advanced atherosclerotic lesion. Smooth muscle cells (SMCs) synthesize the bulk of the extracellular matrix that characterizes this phase of plaque evolution. In response to platelet-derived growth factor (PDGF) released by activated macrophages and endothelial cells, SMCs migrate from the tunica media into the intima, where they proliferate under the influence of various growth factors and secrete extracellular matrix proteins, including interstitial collagen, especially in response to transforming growth factor- β (TGF- β) and PDGF. The **fibrous cap** covering the atherosclerotic plaque, formed during this phase, owes its biomechanical strength to interstitial collagen (types I and III).

Neovascularization arising from the artery's **vasa vasorum** contributes to lesion progression in many ways. First, it provides another entry route for leukocytes into established atherosclerotic lesions. Second, as these neovessels are friable, they can favor focal intraplaque hemorrhage that furnishes one mechanism for the discontinuous increments seen in plaque growth. Hemorrhage in turn generates thrombin, stimulating the release of PDGF from ECs as well as directly stimulating SMC proliferation and cytokine production.

CD40L plays an important role in this phase of atherogenesis. All the main cell types involved in atherosclerosis, including macrophages, T-cells, ECs, SMCs, and platelets, express this proinflammatory cytokine as well as its receptor, CD40. Ligation of CD40 triggers the expression of adhesion molecules and the secretion of numerous cytokines and MMPs involved in extracellular matrix degradation. Importantly, CD40L has a prothrombotic effect by inducing macrophage expression of **tissue factor** (also called thromboplastin, factor III, or CD142), which once exposed to factor VII initiates the coagulation cascade.

Plaque Rupture

Plaque rupture and the ensuing thrombosis commonly cause the most dreaded acute complications of

atherosclerosis. In many cases, the culprit lesion of acute coronary artery thrombosis does not produce a critical arterial narrowing, rendering their identification a priori problematic by use of standard angiographic methods. Decades usually separate the initiation of atherosclerosis with the development of the fatty streak from the final thrombotic stages of this disease. This time lag results in part from the initial compensatory centrifugal remodeling ("compensatory enlargement") of the diseased vessel wall, allowing preservation of blood flow until the stenosis encroaches on >70% of the arterial lumen.

Indeed, it now appears that inflammatory activation rather than the degree of stenosis renders the plaque rupture-prone and precipitates thrombosis and resultant tissue ischemia. Advanced complex atheromata exhibit a paucity of SMCs at sites of rupture, and an abundant macrophage accumulation, key characteristics of plaques that have ruptured and caused fatal coronary thrombosis. Simply put, the fibrous cap is all that stands between coagulation factors in the circulation and the plaque core; the thinner the cap, the greater its propensity to rupture and cause thrombosis. Inflammation can interfere with the integrity of the cap's interstitial collagen by stimulating the destruction of existing collagen fibers while in parallel blocking the creation of new collagen. IFN- γ , secreted by activated T-cells, inhibits basal collagen production by SMCs. T-lymphocytes can also contribute to the control of collagenolysis. CD40L as well as IL-1 produced by T-cells induce macrophages to release interstitial collagenases, including MMPs 1, 8, and 13. Members of the cysteine protease family, such as Cathepsin S, can also participate in plaque evolution and destabilization.

Acute coronary syndromes most often result from a physical disruption of the fibrous cap, either frank cap fracture or superficial endothelial erosion, allowing the blood to make contact with the thrombogenic material in the lipid core or the subendothelial region of the intima. This contact initiates the formation of a thrombus, which can lead to a sudden and dramatic blockade of blood flow through the affected artery. If the thrombus is nonocclusive or transient, it may either be clinically silent or manifest as symptoms characteristic of **unstable angina**. Importantly, if collateral vessels have previously formed, for example, due to chronic ischemia produced by multivessel disease, even total occlusion of one coronary artery may not lead to an acute myocardial infarction.

Pharmacological Intervention

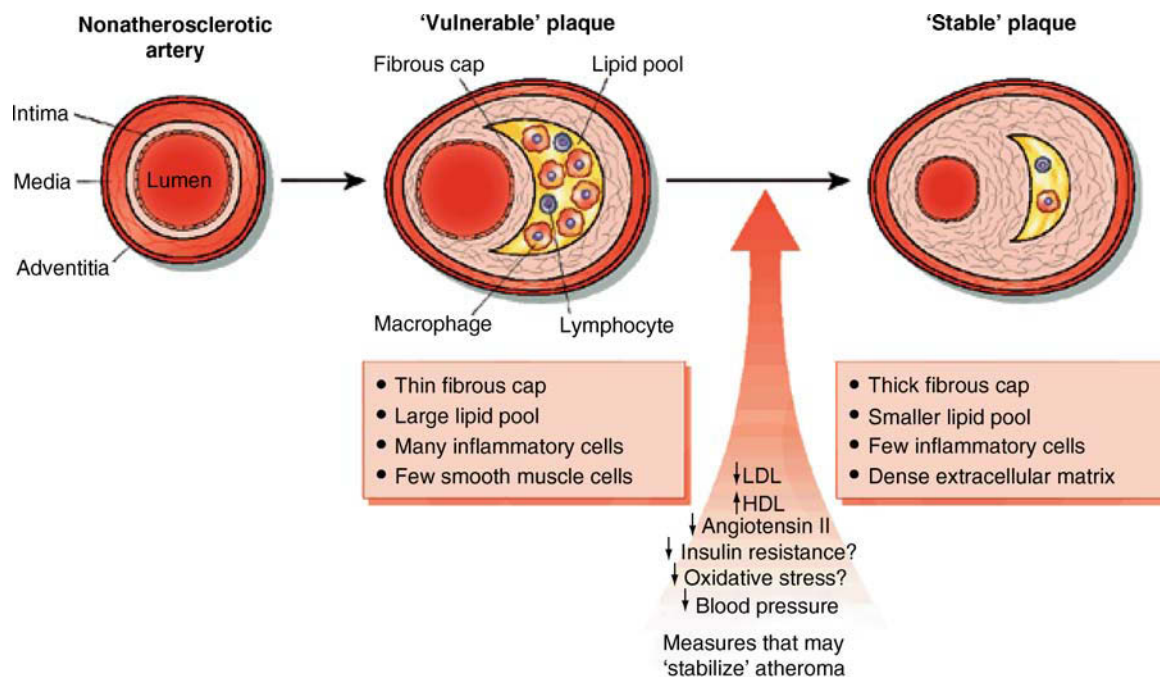
General

Classic risk factors of atherosclerosis must first and foremost be fought with lifestyle interventions such as diet, physical activity, and smoking cessation. Indeed, and although it effectively relieves angina, simply

treating stenotic blood vessels by invasive procedures such as ►[angioplasty](#) or coronary artery bypass grafting has not been shown to prolong life in broad groups of patients.

Pharmacological management of risk factors through antihypertensive, antihyperlipidemic, and antidiabetic therapy coupled with antiplatelet drugs comprise the

next step in the primary and secondary prevention of atherosclerosis, usually instituted together with lifestyle measures [3]. Moreover, medication can in some cases modify or dampen inflammatory processes (Fig. 2). The following review will emphasize the sometimes unforeseen antiinflammatory effects of certain classes of antiatherosclerotic drugs.



Atherosclerosis. Figure 2 Evolution and stabilization of rupture-prone, or "vulnerable," atherosclerotic plaques. The nonatherosclerotic artery (left) has a trilaminar structure; the intima is lined by endothelial cells (ECs) in contact with blood. The underlying media is composed largely of smooth muscle cells (SMCs) and embedded in a dense extracellular matrix. The adventitia, the outermost layer, contains loose connective tissue and nerves. During the early stage in the development of atherosclerosis, the atheroma often grows outward and preserves the caliber of the lumen (middle). Such "compensatory enlargement" or "outward remodeling" explains in part why angiography underestimates the degree of atherosclerosis. Pathological studies have demonstrated that the majority of atheroma that have ruptured and triggered an acute myocardial infarction contain a prominent lipid pool and numerous inflammatory cells, in particular, macrophages. The activated inflammatory cells secrete mediators that thin and weaken the fibrous cap that overlies the lipid-rich core of the lesion by reducing synthesis and increasing degradation of collagen. SMC apoptosis may also contribute to depletion of collagen in the fibrous cap. Activated macrophages express tissue factor, a potent activator of coagulation cascade. Disruption of the thin fibrous cap of such vulnerable plaques causes the direct contact of blood coagulation factors to tissue factor and can trigger occlusive thrombus formation. A new therapeutic goal, stabilization of lesions, aims to reduce the incidence of acute coronary events by influencing the nature of the vulnerable plaque qualitatively or functionally rather than by shrinking the lesion (right). Lowering of LDL (low-density lipoprotein) can reduce cholesterol delivery, and increased HDL (high-density lipoprotein) may enhance cholesterol efflux from the atheroma. LDL reduction and inhibition of angiotensin II signaling may limit oxidative stress (for example, reactive oxygen species production, lipid peroxidation and oxidized LDL accumulation) in atheroma. Future research should evaluate the effects of other therapeutic measures on inflammatory processes mentioned in the text, extracellular matrix metabolism, the thrombotic/fibrinolytic balance, and other functional features of plaque, as well as the effects on lipids and the size of lesions. Conversion of unstable to stable plaques by altering their biological properties should prevent cardiovascular events such as myocardial infarction and stroke by a noninvasive strategy rather than helping in the traditional mechanical approach (bypass surgery, endarterectomy, or angioplasty). (Libby P, Aikawa M (2002) Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. *Nat Med* 8:1257–1262.)

Pharmacotherapy

Statins (3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors)

This lipid-lowering class of drugs efficiently lowers LDL cholesterol levels and reduces cardiovascular events significantly, even in patients with average LDL concentrations. Importantly, statins produce modest effects on the actual degree of arterial stenoses, reemphasizing that the functional state of the atherosclerotic plaque, and not merely its size, determines the propensity of a plaque to precipitate an acute coronary syndrome. This observation suggests that lipid lowering in and of itself may have actions beyond regression of stenoses. Indeed, several inflammatory markers that associate with cardiovascular disease risk, most notably ►C-reactive protein (CRP), decrease in response to statin treatment.

The antiinflammatory effects of statins likely result from their ability to inhibit the formation of mevalonic acid. Downstream products of this molecule include not only the end product, cholesterol, but also several isoprenoid intermediates that covalently modify (“prenylate”) certain key intracellular signaling molecules. Statin treatment reduces leukocyte adhesion, accumulation of macrophages, MMPs, tissue factor, and other proinflammatory mediators. By acting on the MHC class II transactivator (CIITA), statins also interfere with antigen presentation and subsequent T-cell activation. Statin treatment can also limit platelet activation in some assays as well. All these results support the concept that in addition to their favorable effect on the lipid profile, statins can also exert an array of antiinflammatory and immunomodulatory actions.

Peroxisome Proliferator-Activated Receptor Agonists

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor family, and include three forms (α , γ , and δ) [4]. PPAR- α activators (fibrates such as gemfibrozil or fenofibrate) act as triglyceride-lowering/HDL-raising drugs, and PPAR- γ activators (thiazolidinediones such as pioglitazone or rosiglitazone) as insulin-sensitizing agents. The identity of endogenous PPAR ligands remains elusive, with recent work pointing towards a role for lipases such as lipoprotein lipase and endothelial lipase as well as lipoprotein substrates such as very low density lipoprotein (VLDL) and HDL in generating such ligands.

Current evidence suggests that PPAR activation may limit inflammation and hence atherosclerosis. Both PPAR- α and PPAR- γ can reduce T-cell activation, as shown by decreased production of IFN- γ . PPAR- α agonists also repress endothelial VCAM-1 expression and inhibit the inflammatory activation of vascular SMCs, while PPAR- γ agonists repress endothelial chemokine expression and decrease macrophage MMP production.

In humans, biomarker responses to PPAR agonists by and large support possible antiatherosclerotic benefits, although recent clinical trial results have not proven conclusive. Further large-scale trials should help define the role of PPAR agonist therapy on cardiovascular events in at-risk populations.

Angiotensin-Converting Enzyme Inhibitors/Angiotensin-Receptor Blockers

Agents that interfere with signaling of angiotensin II, originally designed to treat hypertension, may also have antiinflammatory effects relevant to atherosclerosis. In addition to its vasoconstrictor and sodium-retaining effects, angiotensin II may act as a proinflammatory cytokine that can elicit VCAM-1 and MCP-1 expression by endothelial cells. Treatment with inhibitors of angiotensin-converting enzyme can reduce signs of inflammation, thereby providing a potential link between antihypertensive and antiatherosclerotic therapy.

Aspirin (Acetylsalicylic Acid)

Randomized trials have clearly established that low-dose aspirin prevents arterial thrombosis, including first myocardial infarction in men, stroke in women, and recurrent vascular events among patients with known atherosclerotic disease. Due to the irreversible inactivation by acetylation of ►cyclooxygenases (COX)-1 and -2, key enzymes in platelet biology, only the generation of new platelets can reverse the antiaggregatory effect of aspirin. The products of COX-1 include thromboxane A₂ (TXA₂), which causes irreversible platelet aggregation and amplifies the platelet response secondary to stimuli such as thrombin, collagen, and adenosine diphosphate (ADP). Importantly, TXA₂ is proatherogenic, and enhances endothelial adhesion molecule expression and subsequent leukocyte-endothelial interaction as well as vascular SMC proliferation. In addition to these specific effects of TXA₂ directly abrogated by aspirin, platelet activation in general leads to the release of proinflammatory cytokines such as CD40L and PDGF, further heightening the inflammatory burden of atherosclerosis.

Future Directions

Vaccination Against Atherosclerosis. Parenteral immunization with oxidatively modified LDL can inhibit experimental atherosclerosis. This protection occurs in parallel with increased titers of antibody specific for the immunogen, and seems to depend mostly on humoral immunity. Although this approach remains unsubstantiated in humans, a vaccination strategy might protect against atherosclerosis and its complications, a proposition that would require rigorous testing in the clinic.

Mast Cell Regulation. Recent experiments have elucidated the deleterious role of mast cell activation in atherosclerotic mice. Atheromata from mast cell

deficient mice in compound mutation with atherosclerosis susceptibility demonstrate decreased lesion size, lipid deposition, T-cell and macrophage numbers, and apoptosis, but increased collagen content and fibrous cap development. Treatment of atherosclerotic mice with the mast cell stabilizer disodium cromoglycate yields similar results. This agent blocks the release of mast cell granular contents such as IL-6 and IFN- γ , which can activate other inflammatory cells present in the atheroma. In addition, treatment with disodium cromoglycate decreases plaque levels of cathepsins and MMPs, yielding plaques with features considered more stable in human lesions.

Given the routine use of mast cell stabilizers in the clinic, for example in the setting of asthma treatment, these preclinical results may stimulate clinical evaluation in humans.

► **Cannabinoid Receptors.** Of the two known cannabinoid receptors, CB2 is expressed predominantly on immune cells. In animals, activation of CB2 can ameliorate chronic inflammation in arthritis as well as experimental allergic encephalopathy. Recent findings suggest that cannabinoids can also benefit atherosclerosis. In atherosclerotic lesions, Δ^9 -tetrahydrocannabinol (THC) blocks IFN- γ secretion by T-cells and reduces macrophage infiltration by inhibiting the expression of the chemokine receptor CCR2. Interestingly, recent findings point toward PPAR- γ as an additional potential target for cannabinoid binding.

Determining whether cannabinoids may enter the list of antiatherosclerotic therapies will require further experimental evidence as well as clinical validation.

Conclusion

The immune response is central to atherosclerosis, from its initiation and development through its thrombotic complications. Indeed, our current understanding of the biology of this scourge differs greatly from the former perspective of atherosclerosis as a lipid storage problem. We now appreciate that the inflammatory activation state of the atheromatous plaque, which ultimately leads to a thin and rupture-prone fibrous cap, rather than the actual degree of luminal encroachment, influences the clinical manifestations of this disease. This enhanced understanding of plaque biology provides new insights into the diverse ways in which atherosclerosis can present clinically and why the disease may remain silent for prolonged periods of time, interrupted by acute complications.

Our new appreciation of the role of inflammation in atherosclerosis shows the way for translation of these novel biological insights to clinical practice, for example by aiding the identification of individuals at risk of adverse cardiovascular events [5]. In this context, inflammatory biomarkers such as CRP merit rigorous consideration for inclusion in risk assessment strategies. In addition, these scientific advances provide a framework

to understand the mechanisms by which lifestyle modifications and certain medical therapies can reduce events by antiinflammatory actions that lead to stabilization of plaques. We can now conceive altering the biology of the atheroma rather than taking a mechanical approach to relieve stenosis by surgical or percutaneous revascularization. Finally, our expanded and deeper appreciation of the biology of atherogenesis should lead to new therapies as well as improved strategies for risk prediction and detection of silent disease, advances that should ultimately improve patient outcomes.

- HMG-CoA-Reductase-Inhibitors
- Peroxisome Proliferator-Activated Receptors (PPARs)
- ACE Inhibitors
- Antiplatelet Drugs

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Atherosclerotic Plaques

Atherosclerotic plaques are lesions in the arterial vessels which arise during the process of atherogenesis. Most cases of acute heart attacks are caused by rupture of an atherosclerotic plaque.

- Atherosclerosis
- HMG-CoA-Reductase-Inhibitors
- Antiplatelet Drugs
- ACE Inhibitors

Atopy

Atopy is the propensity to develop allergic reactions mediated by immunoglobulin E.

- Allergy
- Leukotrienes

ATP

Adenosine triphosphate (ATP) is a purine nucleotide involved in extracellular signalling, as well as acting as an intracellular energy source.

► Purinergic System

ATP-binding Cassette Transporter Superfamily

ATP-binding cassette (ABC) transporters (proteins) are characterized by having so-called ATP-binding cassette domains. ABC proteins function as pumps, channels, and channel regulators (receptors). They have multiple membrane-spanning segments and nucleotide-binding folds (domains) (NBFs or NBDs) in the cytoplasmic side, which contain highly conserved Walker motifs and an ABC signature sequence. Cystic fibrosis transmembrane conductance regulator, *P*-glycoprotein, canalicular multispecific organic anion transporter, and sulfonylurea receptor are typical ABC proteins.

- ABC Transporters
- MDR-ABC Transporters
- Multidrug Transporter
- Lysophospholipids
- Sterol Transporters
- ATP-dependent K^+ Channel

ATP-dependent K^+ Channels

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Synonyms

ATP-sensitive K^+ channel; ATP-regulated K^+ channel; K_{ATP} channel; SURx/Kir6.x channel

Definition

Key feature of K_{ATP} channels is their responsiveness to changes in intracellular ATP and ADP providing a

means to couple electrical activity to cellular metabolism in many excitable cells (e.g., pancreatic beta-cells, neurons, and cardiac myocytes). K_{ATP} channels are assembled from alpha (►inward rectifier K^+ channel, Kir6.x) and beta (sulfonylurea receptor, SUR) subunits with obligate hetero-octameric stoichiometry (SUR/Kir6.x)₄. Four alpha subunits form the ion-conducting pore, which is surrounded by four beta subunits with receptor sites for drugs and ADP (Figs. 1a and b).

Basic Characteristics Assembly

Trafficking from the endoplasmic reticulum to the cell surface is regulated by arginine-rich RKR motifs on both subunits in combination with a C-terminal signal on SUR. These motifs insure correct assembly of the complex and channel surface expression. ►14-3-3-proteins seem to be involved in this process. Shortly after synthesis, SUR1 and Kir6.2 presumably dimerize, protecting Kir6.2 from degradation. The SUR1/Kir6.2 dimers then assemble the octameric complex. SUR and Kir6.x subunits are the products of two pairs of genes on human chromosomes 11 and 12. *ABCC8* encodes for SUR1 (OMIM 600509) and lies in position 11p15.1, 5 kb upstream of the gene for Kir6.2 (*KCNJ11*, OMIM 600937). Encoding for SUR2A and SUR2B, *ABCC9* lies in position 12p12.1, 26.2 kb upstream of the gene for Kir6.1 (*KCNJ8*; OMIM 600935). SUR2A and SUR2B result from differential splicing of the terminal exon of *ABCC9*.

Tissue Distribution

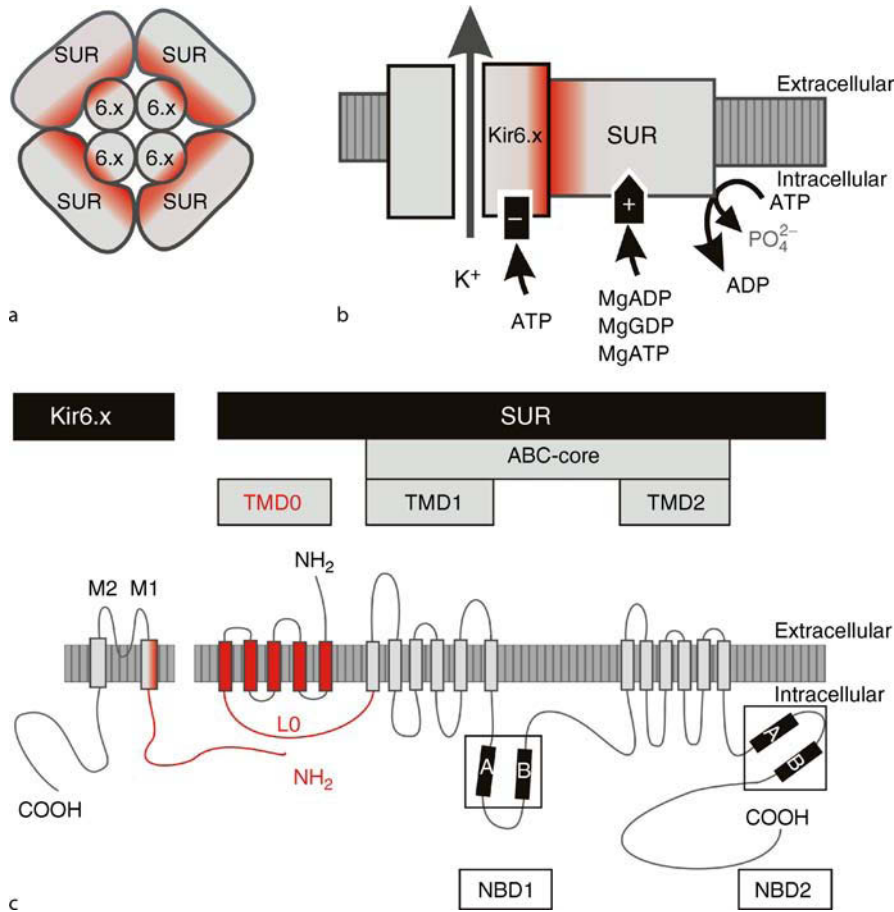
SUR1 assembles with Kir6.2, and (SUR1/Kir6.2)₄ channels are broadly distributed in the neuroendocrine system. (SUR2A/Kir6.2)₄ constitutes the K_{ATP} channels found in cardiac and skeletal muscle cells, and (SUR2B/Kir6.1)₄ those in vascular and nonvascular smooth muscle.

Kir6.x

Transmembrane helices M1 and M2 of the four Kir6.x subunits interact to form the ion-conducting pore of the channel. In analogy with a proposed mechanical mechanism for regulation of Kv channels (►voltage-gated K^+ channels), gating is presumably mediated by repositioning M1 and M2 through movement of a submembrane helix termed “the slide helix” (Fig. 2). The receptor site for inhibitory ATP has been localized at the interface between adjacent Kir6.x subunits, approximately 2 nm below the outer surface of the membrane.

SURs

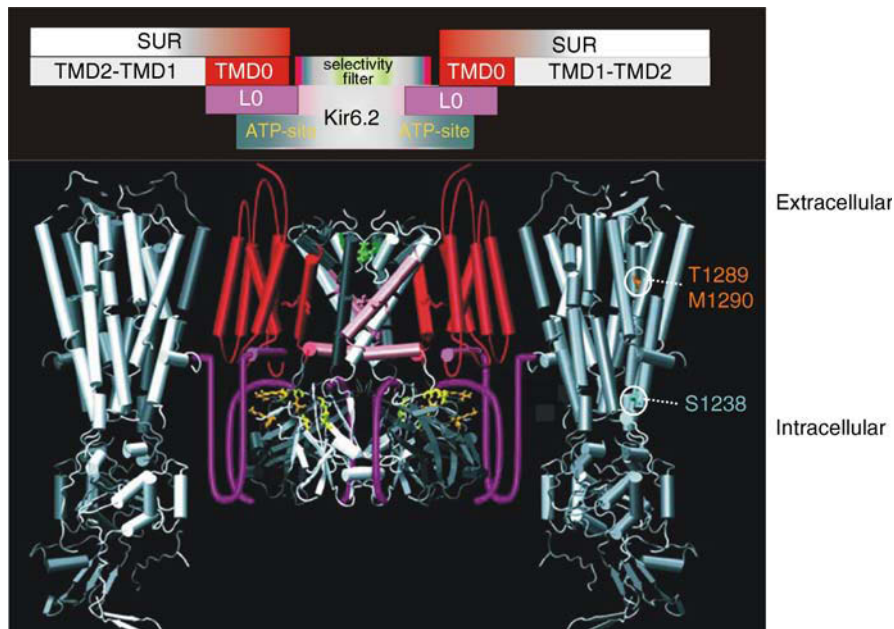
SURs are members of the ATP-binding cassette (ABC) family of proteins (►ABC transporters) with a typical ABC “core” consisting of two bundles of six



ATP-dependent K⁺ Channels. Figure 1 (a) Tetradimeric architecture. K_{ATP} channels are assembled from alpha (Kir6.x) and beta (SUR) subunits with obligate hetero-octameric stoichiometry (SUR/Kir6.x)₄. Four alpha subunits form the ion-conducting pore, which is surrounded by four beta subunits. (b) Regulation by nucleotides. The receptor site for inhibitory ATP resides on the pore-forming Kir subunit, while activatory nucleotides bind to a separate nucleotide site on SUR. The NBDs of SUR bind and hydrolyse ATP. (c) Two-dimensional topology model of K_{ATP} channels. A and B symbolize the Walker A and B consensus motifs in the two NBDs, respectively; TMD: TransMembrane Domain, NBD: Nucleotide-Binding Domain, L0: intracellular linker between TMD0 and TMD1. Putative contact regions between Kir6.x (parts of M1 plus NH₂-terminus) and SUR (TMD0 plus L0) depicted in red.

transmembrane helices (TMD1 and 2) and two nucleotide-binding domains (NBD1 and 2) (Fig. 1c). Similar to several other ABCC proteins (i.e., ABCC1, 2, 3, 6, and 10), SURs have an additional amino terminal module that consists of a bundle of five transmembrane helices (TMD0). These additional helices are connected to the core via an intracellular linker termed “L0” (Fig. 2). In *ABCC8* and *9*, TMD0-L0 is the principal domain interacting with the Kir subunit. The SUR NBDs contain the canonical phosphate-binding Walker A and B motifs, the Q-loop, the signature sequence, and the H-loop, hallmarks of the ABC family. The SURs were among the first ABC proteins recognized to have degenerate, nonsymmetric NBDs with a noncanonical signature sequence, FSQGQ versus LSGGQ, in NBD2 and an aspartate (D) in place of the usual glutamate (E)

adjacent to the highly conserved D in the Walker B motif. In analogy with other ABC proteins, ATP binding and hydrolysis are expected to drive dimerization of the SUR NBDs and produce concomitant rearrangements of TMD1 and TMD2. Typically coupled to substrate transport, SURs appear unique by transducing these conformational changes in modulation of channel gating. TMD0 plus subsequent intracellular loop L0 appear to be critical in this process. Analogous to the voltage sensor in Kv2.1 channels, L0 presumably affects gating through direct interaction with the Kir “slide helix” (Fig. 2). Localization of the site of action for activatory Mg-nucleotides (e.g., MgADP, MgATP, MgGDP) on SURs is still controversial. Cytosolic [MgADP] might enhance channel activity by lowering the off-rate of MgADP from NBD2 through product



ATP-dependent K⁺ Channels. Figure 2 Three-dimensional homology model of K_{ATP} channels. *Green*: pore selectivity filter, horizontal *pink* cylinder: the “slide helix”, bundle of five *red* cylinders: TMD0, *purple*: L0 + distal parts of Kir amino termini, *gold* and *yellow*: residues involved in ATP-binding, S1238: critical for SU-binding, T1286 plus M1290 critical for K_{ATP}CO-binding. (Panel modified from [3]).

inhibition. Alternatively, evidence has been presented that Mg-nucleotides exert their activatory effect not by interaction with the catalytic NBD sites but by binding to a separate nucleotide site mainly formed by TMD2.

Role in Pancreatic Beta-Cells

The role of K_{ATP} channels in cellular function is best understood in pancreatic beta-cells (Fig. 3). Insulin release is triggered by a rise of intracellular [Ca²⁺]_i that results from Ca²⁺ influx through voltage-gated Ca²⁺ channels in the beta-cell plasma membrane. Under low serum [glucose], the Ca²⁺ channels are shut because the membrane is held hyperpolarized by potassium outward currents through K_{ATP} channels. When blood glucose levels are elevated, glucose is rapidly equilibrated across the beta-cell membrane via the GLUT2 transporter (▶ [glucose transporters](#)). In the next rate-limiting step, it is phosphorylated by glucokinase. Subsequent glycolysis and mitochondrial metabolism leads to changes in intracellular concentrations of adenine nucleotides and closure of K_{ATP} channels. This produces a membrane depolarization that opens ▶ [voltage-dependent Ca²⁺ channels](#), initiating beta-cell electrical activity and Ca²⁺ influx. The subsequent rise in [Ca²⁺]_i triggers insulin release. In resting beta-cells, 98% of plasmalemmal K_{ATP} channels are closed and hence, insulin release is induced by reducing flux through residual 2%. Cytosolic free [ATP] not below 1mM, however, predicts that even in the resting state

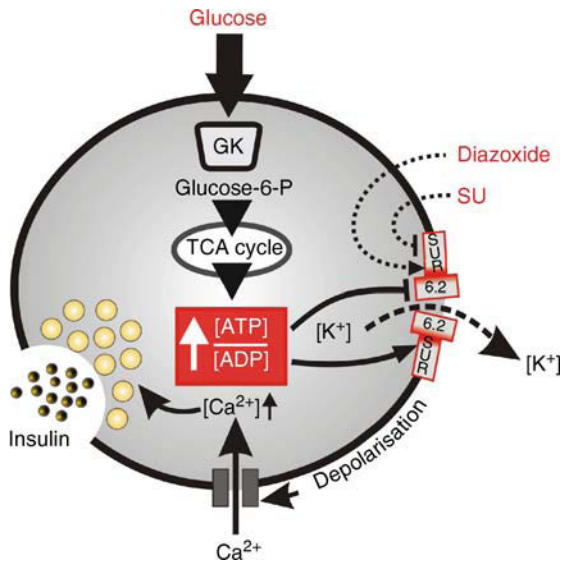
channel activity should be completely suppressed and a definite answer to this “ATP-paradox” is still pending. One possibility is that the concentration–inhibition curve is shifted to higher ATP levels through stimulatory effects of Mg-nucleotides. Alternatively, [ATP] in the immediate vicinity of the Kir6.2 binding pocket might be much lower than estimated by measurement of total cytosolic ATP.

Modulators

Phosphoinositides, long-chain acyl coenzyme A (CoAs), G proteins, and phosphorylation have been shown to modulate K_{ATP} function. The physiologic role of these modulators, however, has still to be established. Lower pH activates K_{ATP} channels by reducing their sensitivity to inhibitory ATP and may play a role in the regulation of vascular tone during hypercapnic acidosis. Zn²⁺ activates SUR1/Kir6.2 K_{ATP} channels via binding to two histidines on the extracellular face of SUR1. Zn²⁺ is present in high concentrations in various regions of the CNS. In pancreatic islets it is coreleased with insulin and may play an autocrine role or serve to attenuate glucagon release from alpha cells.

Neuroprotection

Substantia nigra pars reticulata is the area with highest neuronal activity and metabolic rate in the brain. This region also shows highest expression rates of SUR1/Kir6.2 channels. These K_{ATP} channels are present



ATP-dependent K⁺ Channels. Figure 3 Stimulation of insulin secretion in the beta-cell. A rise in extracellular [glucose] increases phosphorylation of glucose by glucokinase (GK), the rate-limiting enzyme for the metabolism of glucose in the beta-cell. Glycolysis results in conversion to pyruvate, which preferentially enters the mitochondria and fuels the tricarboxylic acid (TCA) cycle. The resultant increase in the [ATP/ADP] ratio leads to closure of K_{ATP} channels, depolarization of the plasma membrane, opening of voltage-gated Ca²⁺ channels, and an increase of the cytosolic Ca²⁺ concentration, which triggers the exocytosis of insulin. Sulfonylureas (SUs) inhibit the K_{ATP} channel and initiate the same chain of events, whereas diazoxide activates the channel, hyperpolarizes the beta-cell, lowers cytosolic Ca²⁺, and inhibits insulin secretion. Glucose-6-P, glucose-6-phosphate.

throughout all regions of the CNS and confer neuroprotection by rapid hyperpolarization and thus minimization of energy consumption during metabolic stresses, like hypoxia and ischemia. In substantia nigra, SUR1/Kir6.2 channels confer a specific sensor function for ATP depletion thus enabling an early generalized protective response. Fast electrical silencing of GABAergic neurons within substantia nigra results in prevention of generalized seizure by disinhibition of distant projection areas (e.g., ventral thalamic nuclei, nuclei superior colliculi, pedunculopontine nucleus).

Central Glucose Homeostasis

Central control of glucose homeostasis critically depends on the brain's ability to sense extracellular [glucose]. Within hypothalamus at least two types of neurons were identified which are presumably involved in this process. They are either glucose excited or glucose inhibited. Both types of neurons appear to be involved in the control of feeding, hepatic gluconeogenesis,

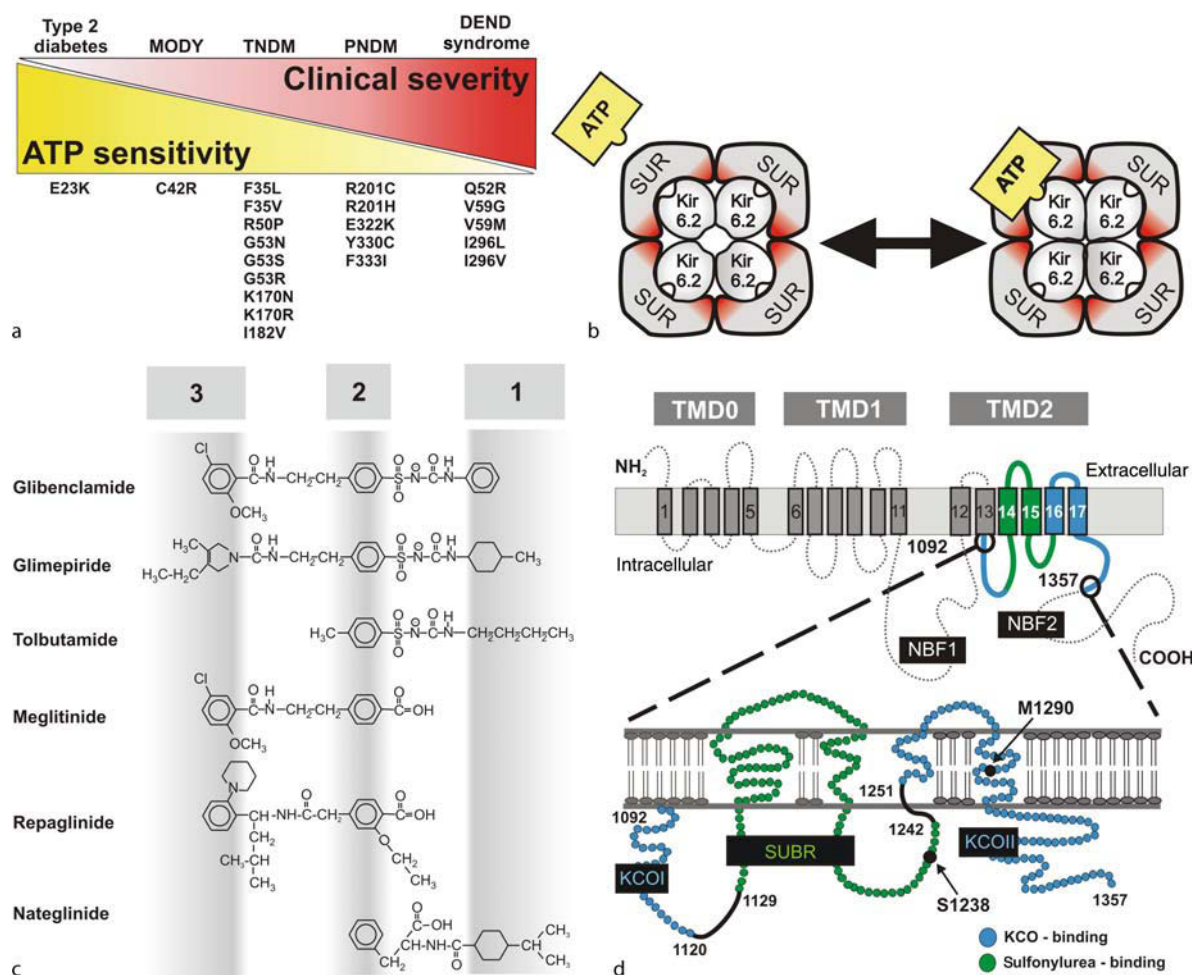
and glucagon and epinephrine secretion. In glucose excited neurons, the sensing mechanism appears to be based on SUR1/Kir6.2 channels, analogous to that in pancreatic beta-cells. Consistently, glucose sensing was lost in Kir6.2^{-/-} mice or intracerebral application of glibenclamide.

Hyperinsulinemic Hypoglycemia

Mutations in SUR1 and Kir6.2 are an established cause of ▶hyperinsulinemic hypoglycemia of infancy (HHI), characterized by excessive insulin release independent from blood [glucose]. Most cases of HI are sporadic and the disease may result from homozygous or heterozygous mutations. Mutations in SUR1 (*ABCC8*) are the most common cause of HI, accounting for almost 50% of cases. More than 100 mutations have been described, distributed throughout the gene. Class I mutations are characterized by loss of K_{ATP} channels in the plasma membrane. This may result from impaired SUR1 synthesis, abnormal SUR1 maturation, defective channel assembly, or faulty surface membrane trafficking. Class II mutations impair the ability of MgADP to stimulate channel activity. In general, class II mutations cause a milder phenotype because residual channel activity remains. They can also be causative for leucine-sensitive HI (e.g., R1353H). Most class II mutations reside in the NBDs of SUR1. To date in Kir6.2 (*KCNJ11*) five mutations have been identified that cause HI. They also act by reducing, or abolishing K_{ATP} channel activity in the surface membrane, thus inducing permanent beta-cell depolarization, uncontrolled insulin release, and hypoglycemia.

Neonatal Diabetes

Neonatal ▶diabetes mellitus (NDM) is characterized by hyperglycemia within the first 6 months of life. It is a rare disorder affecting 1 in 400,000 live births, and it may be either transient (TNDM) or permanent (PNDM). Approximately 50% of PNDM cases result from heterozygous gain-of-function mutations in Kir6.2 [2]. To date, more than 20 mutations in *KCNJ11* have been reported to cause NDM. They form striking clusters in the putative ATP-binding site (Fig. 2) and the cytosolic pore of the channel. Mutations within residues, V59 and R201, show highest frequency. A series of gain-of-function mutations cause a range of phenotypes with increasing severity (Fig. 4a). In most cases, PNDM is observed without additional symptoms. These patients show weak insulin release in response to i.v. glucose but may respond to sulfonylureas. Other mutations cause more severe phenotypes with delayed speech, walking, and muscle weakness in addition to neonatal diabetes. A third class of mutations (e.g., Q52R, V59G) produce the severe DEND syndrome (developmental delay, epilepsy, and neonatal diabetes), characterized by marked developmental retardation, muscle weakness, epilepsy, and dysmorphic



ATP-dependent K⁺ Channels. Figure 4 (a) Genotype–phenotype correlation for gain-of-function mutations in Kir6.2. (b) Stoichiometry of ATP-induced channel closure. (c) Structure of sulfonylurea and nonsulfonylurea hypoglycemic agents. Grey: lipophilic centers 1–3. (d) Regions within SUR critical for drug binding.

features besides hyperglycemia. Mutations that cause TNDM or maturity onset diabetes of the young (MODY) are also found. All PNDM mutations studied to date act by reducing the potency of ATP to close the channel and this effect correlates quite well with clinical phenotype. Channels are closed if just one of the four sites for inhibitory ATP is occupied and thus, strongly reduced ATP sensitivity is only observed in channels with four mutated subunits (Fig. 4b). Therefore, a marked increase in open probability is found in no more than 6% of the channels of heterozygotes. This effect, however, is sufficient to completely abolish insulin release (see earlier). In extrapancreatic tissues expressing Kir6.2 (skeletal muscle, cardiac muscle, and neurons throughout the brain) dependence on open probability appears less critical, explaining lack of additional symptoms in heterozygote patients with PNDM mutations (e.g., Q52R, V59G, and I296L). Mutations causing the severe DEND phenotype affect ATP sensitivity by increasing spontaneous open probability of the channel.

Here, one mutated subunit is sufficient to strongly reduce ATP sensitivity thereby inducing additional symptoms in extrapancreatic tissues. To date two mutants within SUR1 are known (I1424V and H1023Y), resulting in hyperactive channels and PNDM.

Type 2 Diabetes Mellitus

Large-scale association studies indicate that a common variant (E23K) in Kir6.2 is strongly associated with an enhanced susceptibility to type 2 diabetes [1]. Although the effect is small, the high prevalence of the K allele (34%) makes this a significant population risk. The E23K polymorphism confers an increase in intrinsic open probability, with a consequent reduction in ATP sensitivity, and enhanced activation by Mg-nucleotides and long-chain acyl-CoAs. In glucose-tolerant subjects it was demonstrated to be associated with reduced insulin and increased glucagon secretion. Polymorphisms in HNF1alpha, HNF4alpha, and glucokinase (MODY), and in genes involved in mitochondrial metabolism, have also

been associated with an increased risk of type 2 diabetes. They might influence disease susceptibility by impairing metabolic regulation of K_{ATP} channel activity.

Arrhythmias and Heart Failure

Data from Kir6.2 knockouts suggest that SUR2A/Kir6.2 channels in the heart are required for the adaptive response to acute stress and chronic hemodynamic load. Thus, deficiency is presumed to be associated with stress-induced arrhythmias, defective structural remodeling, calcium-dependent maladaptation, and predisposition to heart failure. Consistently, two mutations were identified in exon 38 of the human *ABCC9* gene that resulted in dilated cardiomyopathy. A male who was diagnosed at age 55 and died from heart failure at age 60, had a 3-bp deletion followed by a 4-bp insertion (4570–4572delTTAinsAAAT) causing a frameshift at leu1524 and introducing four anomalous terminal residues followed by a premature stop codon. The patient died at age 60 and had no family history of dilated cardiomyopathy. A female who was diagnosed at age 40 had at 1513 an alanine to threonine substitution. Her father was diagnosed at age 54 and died at age 55 of heart failure. All three individuals had ventricular tachycardia with normal coronary angiography. Both mutations were not identified in unrelated healthy controls. The C-terminus of SUR proteins contributes to K_{ATP} channel trafficking, and the frameshift and missense SUR2A mutants, reconstituted with Kir6.2, had reduced expression in the plasma membrane. Yet, mutant K_{ATP} channel complexes formed functional channels with intact pore properties. Residues ala1513 and leu1524 flank the C-terminal beta-strand in close proximity to the signature Walker A motif, required for coordination of nucleotides in the catalytic pocket of ATP-binding cassette proteins. Replacement of ala1513 with a sterically larger and more hydrophilic threonine residue or truncation of the C-terminus caused by the frameshift would disrupt folding of the C-terminal beta-strand. Nucleotide-induced K_{ATP} channel gating was aberrant in both channel mutants, suggesting that structural alterations induced by the mutations distorted ADP-dependent pore regulation through SUR2A.

Prinzmetal Angina

Mice lacking the Kir6.1 gene had a phenotype resembling Prinzmetal angina. Spontaneous vasospasms of hypercontractive coronary arteries were associated with ST elevation, atrioventricular block, and a high rate of sudden death. Results presume *KCNJ8* and *ABCC9* as candidate genes for human disease.

Drugs

K_{ATP} channels are the targets for two classes of therapeutic agents, hypoglycaemic drugs like glibenclamide or nateglinide and potassium channel openers like

diazoxide or P1075. In pancreatic beta-cells glibenclamide and analogous substances stimulate insulin secretion by closing K_{ATP} channels while diazoxide shifts the plasma membrane potential toward the potassium equilibrium potential, thus reducing electrical activity and inhibiting insulin release.

Sulfonylureas and Analogs

Hypoglycemic drugs comprise sulfonylureas (e.g., tolbutamide, glibenclamide, glimepiride) and nonsulfonylureas (e.g., meglitinide, repaglinide, nateglinide) (Fig. 4c). Except for repaglinide these compounds are weak organic acids (pK_a values 3.1–6.8). Their protonated species diffuse rapidly across the plasma membrane, dissociate and gain access to their binding site via the cytoplasmic face of SURs. Structure–activity data suggest that high affinity SUR1 selective ligands require three lipophilic centers within their molecule. Center 1 (e.g., a cyclohexyl group in glibenclamide or a cyclohexane substituted with an isopropyl group in nateglinide) is the major determinant of selectivity toward SUR1. Drugs lacking this part of the molecule (e.g., meglitinide) show same affinity for SUR1 and SUR2 isoforms.

Localization of the Binding Site

Affinity of glibenclamide for SUR1 (*K_D* 0.72 nM) is 350 higher than that for SUR2A/B. Based on this difference a 114 amino acid segment within TMD2 (SUBR; C1129–T1242) was identified as putative part of the receptor binding site (Fig. 4d). Substitution of S1238 within this region by Y strongly reduced glibenclamide affinity without affecting binding of meglitinide. Thus this residue was presumed to contribute to interaction with the center 1 region of the ligands. Centers 2 and 3 seem necessary for interaction with either of the SUR isoforms. Both L0 and the proximal amino terminus of Kir6.2 are likely to participate in formation of this part of the binding pocket.

Mechanism of Action

Every SUR subunit carries one site for inhibitory drugs, and hence there are four of these sites per channel complex. Analogous to ATP-induced inhibition (see earlier) occupation of just one of these sites is sufficient to close the channel. This effect is mediated by equalizing Mg-nucleotide-induced channel activation.

Therapeutic Use

Sulfonylureas and glinides are largely used to control hyperglycemia in the treatment of T2DM. In addition many patients with NDM show sulfonylurea sensitivity. Thus, in these patients the drugs appear as an alternative to insulin injections. To date, good glycemic control has been reported for several patients. In DEND patients, therapy with sulfonylureas and analogs might prove

helpful in the control of symptoms resulting from enhanced channel activity in extrapancreatic tissues (see earlier, paragraph “Neonatal Diabetes”).

K_{ATP} Channel Openers

K_{ATP} channel openers (K_{ATP}COs) comprise a structurally diverse group of compounds (e.g., pinacidil, P1075, levromakalim, rilmakalim, minoxidil sulphate, nicorandil, diazoxide) which exert their activatory effect on the channels by interaction with SURs. Some K_{ATP}COs are SUR1-selective (e.g., NN414), most, however, show clear selectivity for SUR2 isoforms (e.g., *K_D* of P1075 for SUR2B = 11 nM and for SUR1 = 1.02 mM).

Localization of the Binding Site

The cytosolic loop between TMs 13 and 14 (KCO I) and TMs 16–17 (KCO II) were identified as critical for K_{ATP}CO binding to SURs (Fig. 4d). T1286 and M1290 appeared to be particularly important. Close local association of sulfonylurea and KCO binding regions might represent the structural basis for negative allosteric coupling of the sites.

Mechanism of Action

K_{ATP}COs do not bind to SURs with defect NBDs indicating that affinity of the receptor site depends on the catalytic state. Similarly, defective NBDs equalize Mg-nucleotide-induced channel activation and are thus causative for HI (see earlier). In analogy to sulfonylureas K_{ATP}CO-induced channel activation is mediated by interaction with one of the four sites per tetradimeric complex. Occupation of additional sites did not induce stabilization of the open state.

Therapeutic Use

Diazoxide, pinacidil, cromakalim, minoxidil sulfate, and nicorandil have been extensively studied. Although there is a broad spectrum of potential therapeutic applications (e.g., hypoglycemia, hypertension, arrhythmias, angina pectoris, cardiac ischemia, asthma), at present none of these drugs is in widespread clinical use. Nicorandil is approved for the treatment of coronary artery disease. Minoxidil sulfate serves for the therapy of severe hypertension that responds poorly to other hypertensive medications. In addition, it is applied topically to stimulate hair growth. Diazoxide is used to suppress excessive insulin secretion in forms of hyperinsulinism (HI) with responsive K_{ATP} channels. HI caused by mutations in glucokinase (*GCK*), glutamate dehydrogenase (*GLUD1*), or short-chain l-3-hydroxyacyl-CoA dehydrogenase (*SCHAD*) responds well to diazoxide. Severe forms of HI caused by mutations in SUR1 or Kir6.2, however, are refractory to diazoxide and require subtotal pancreatectomy (see earlier, paragraph Hyperinsulinemic Hypoglycemia). In some HI SUR1 mutants

(e.g., R1349H) diazoxide was shown to act as chemical ▶**chaperone** and correct defective surface trafficking. This effect might prove valuable in future HI therapy.

- ▶ K⁺ Channels
- ▶ Voltage-gated K⁺ Channels
- ▶ Inward Rectifier K⁺ Channels
- ▶ Diabetes Mellitus
- ▶ Antidiabetic Drugs other than Insulin

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ATP-powered Pump

- ▶ Table appendix: Membrane Transport Proteins

ATP-regulated K⁺ Channel

- ▶ ATP-dependent K⁺ Channel

ATP-sensitive K⁺ Channel

- ▶ ATP-dependent K⁺ Channel

Atrial Fibrillation

Cardiac arrhythmia with a rapid and irregular activity in different areas within the upper chambers (atria) of the heart is also known as atrial fibrillation.

- ▶ Cardiac Glycosides
- ▶ Antiarrhythmic Drugs

Atrial Natriuretic Peptide

The atrial natriuretic peptide (ANP) belongs to a family of hormones that have structural similarity and some biological actions in common, such as natriuresis and haemoconcentration. It is synthesized and secreted by the cardiac atrium in response to increased atrial pressure. ANP is believed to act physiologically in an opposing manner to AVP.

- ▶ Guanylyl Cyclases
- ▶ Vasopressin/Oxytocin

Attention Deficit Hyperactivity Disorder

Synonyms

ADHD

Definition

ADHD is a disorder of childhood. Key features of Attention Deficit Hyperactivity Disorder (ADHD) – with a highly differing prevalence of 0.1–10% – are distractibility and difficulties in sustaining attention and focusing on a task. These symptoms are associated with impulsiveness, regardless of consequences. Comorbidity is high; boy to girl ratio is 4:1. The diagnosis is made using the diagnostic and statistical manual of the American Psychiatric Society (DSM-IV). There is currently no biological test to confirm the diagnosis. The DSM-IV assessment distinguishes between three subtypes of ADHD where (i) inattentiveness, (ii) hyperactivity-impulsivity, or (iii) a combination is prevalent. The period of formal schooling is usually the most difficult life phase for persons with ADHD. At later stages, these individuals often find occupational or educational niches that accommodate their behavioral and cognitive idiosyncrasies. About 50% of children with ADHD continue to manifest dysfunctional symptoms into adulthood. There is relatively poorer occupational and educational outcome, greater psychiatric comorbidity than in control subjects, and significantly higher rates of socialization disorders and substance use disorders in adults in whom ADHD symptoms persist.

The most common treatment of ADHD is pharmacological. Psychostimulant drugs such as methylphenidate and amphetamine or atomoxetine, an inhibitor of the noradrenaline transporter can be prescribed. These agents elicit the non-exocytotic release of

noradrenaline, serotonin, and dopamine via their cognate cell surface transporters. They have considerable abuse liability.

- ▶ Neurotransmitter Transporters
- ▶ Psychostimulants

AUC

AUC is the area under the (drug) concentration time curve.

- ▶ Area under the Curve
- ▶ Pharmacogenetics

Autacoid

Autacoids are literally ‘self-medicating agents’ that are liberated from or produced by cells in response to a stimulus. They differ from hormones in that they usually act locally after release, rather than reaching their target organ via the bloodstream.

- ▶ Emesis
- ▶ Prostanoids
- ▶ Histaminergic System

Autoantigen

Structures expressed in the organs of an individual against his own immune system can mount an immune response. Autoantigens can be organ specific (e.g. insulin) or present in all cells (e.g. DNA).

- ▶ Autoimmune Disease
- ▶ Immune Defense

Autocrine

A mode of action of a molecular messenger such as a cytokine or hormone in which the molecule binds to receptors on, and affects the function of, the cell type that produced it.

Autoimmune Disease

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Definition

The immune system provides powerful defense mechanisms against infective or toxic agents. Its hallmark is the high specificity with which harmful noxes can be recognized and discriminated. Although usually prevented, the immune system sometimes can react against components of the own body resulting in a great variety of disorders, the autoimmune diseases.

Basic Mechanisms

To survive in an environment which contains a plethora of life threatening infectious agents and other harmful noxes, higher organisms have developed special defense mechanisms contained in the immune system ($\rightarrow$ immune defense). In vertebrates, it is based on two arms. The phylogenetically older part (which can be traced back to unicellular organisms) in human beings is represented by the phagocytic leukocytes such as the monocytes/macrophages and the various granulocytes. These cells possess a restricted number of receptors, including the Toll like receptors, which recognize structural patterns common in different strains of viruses, bacteria, fungi or parasites. The major defense mechanisms consist in phagocytosis and subsequent intracellular enzymatic degradation. This is supported by the secretion of many mediators which together form an inflammatory response ($\rightarrow$ inflammation). This “innate immunity” is present from birth, and its reaction remains identical throughout life.

Later in evolution, the second arm evolved, built up by lymphocytes. These cells exist in two main classes: B-lymphocytes which differentiate in the bone marrow, and T-lymphocytes which differentiate in the thymus. B-lymphocytes secrete antibodies into the body fluids. T-lymphocytes exert cellular defense mechanisms, such as killing of e.g. virus infected cells. They also play a central role in regulating immune reactions ($\rightarrow$ immune defense). The novel acquisition of this “adaptive immunity” consists of two connected properties: specificity and memory. Lymphocytes can recognize and distinguish an extremely large number of molecular structures (termed in immunology antigens); in human beings this ranges up to about 10^{18} of which approximately 10^8 are realized in an individual. Each lymphocyte carries only receptors of a single specificity. When mounting an immune response – e.g. against an

invaded bacterium – cells recognizing their respective antigen proliferate and acquire their specific functions implicated in the defense mechanisms. Part of these antigen specific cells develop into memory cells, which possess the capacity to react faster and even more efficient against the same antigen (or the infective bearing this antigen). It is this property that allows an individual to adapt to an environment containing specific infectious agents.

The adaptive immune system contains effector mechanisms of its own such as cytotoxic T-lymphocytes. In most instances, however, it relies on the mechanisms of the innate immune system. Not only directs it the cells of the innate system specifically to the antigen, but also strongly enhances their capacity to deal with harmful noxes. Thus B-lymphocytes release antibodies – secreted forms of their antigen receptor – which bind to their antigens and thereby recruit and activate the phagocytic cells, and also soluble effector systems such as complement. T-lymphocytes in contact with their cognate antigens secrete ($\rightarrow$) cytokines which boost the activity of the cells of the innate immune system.

Self Tolerance

The antigen receptors are proteins. A simple calculation shows that the antigen receptor cannot be inherited. More than 10^8 specificities, i.e. different proteins by far exceed the number of the 25–30,000 genes existing in human beings. The antigen receptors thus must be generated during the development of individual T- or B-lymphocytes. Both antigen receptors are composed of two chains. Although B- and T-cell receptors are different proteins, their generation follows closely related rules. For each of the respective receptor chains there exist inherited in the germ line two to three clusters containing up to less than 100 gene segments. These are assembled in the developing lymphocyte by somatic recombination to form the antigen binding part of the receptor chain resulting in a large combinatorial diversity. This is much increased by the insertion of random nucleotides at the junction of two gene segments during the somatic recombination.

It is clear that generated by such a random process the initial repertoire of antigen specificity in all individuals of human beings should be quite similar. This implies that initially in each individual there develop lymphocytes which recognize antigens of this very individual, i.e. **▶autoantigens**.

As most humans are healthy, at least do not destroy their own organs, there must exist mechanisms which eliminate the **▶autoreactive lymphocytes**. For T-lymphocytes this happens in the thymus. When cells with functional antigen receptors develop, those which react with self (= auto) antigens present in the thymus with high affinity undergo apoptosis (= programmed cell death). An elegant mechanism governed by the

transcriptional regulator AIRE (autoimmune regulator) provides that in special epithelial cells of the thymus tissue antigens of all organs of the body are ectopically (i.e. in an unusual place!) expressed. Those cells which leave the thymus to become the functional T-lymphocytes of the body thus exhibit the “central ►self tolerance” by not reacting with ►autoantigens in the healthy individual. Similar mechanisms are also operative for B-lymphocytes in the bone marrow. The self tolerance predominantly is secured by T-lymphocytes.

The mechanisms of the central self tolerance implies that T- and B-lymphocytes bearing receptors which recognize autoantigens with low affinity are present in the body. These cells are held under control by the mechanisms of peripheral tolerance. Several mechanisms contribute to this (Fig. 1). That T-lymphocytes are activated requires the binding of the antigen to its antigen receptor (“signal 1”), but also accessory signals (termed “signal 2”) are necessary. The antigen (peptide) has to be presented to the T-lymphocytes bound to molecules of the major histocompatibility complex (MHC); to the central T-helper cells bound to MHC class II molecules present predominantly on dendritic cells, macrophages, or B-lymphocytes (→ immune defense).

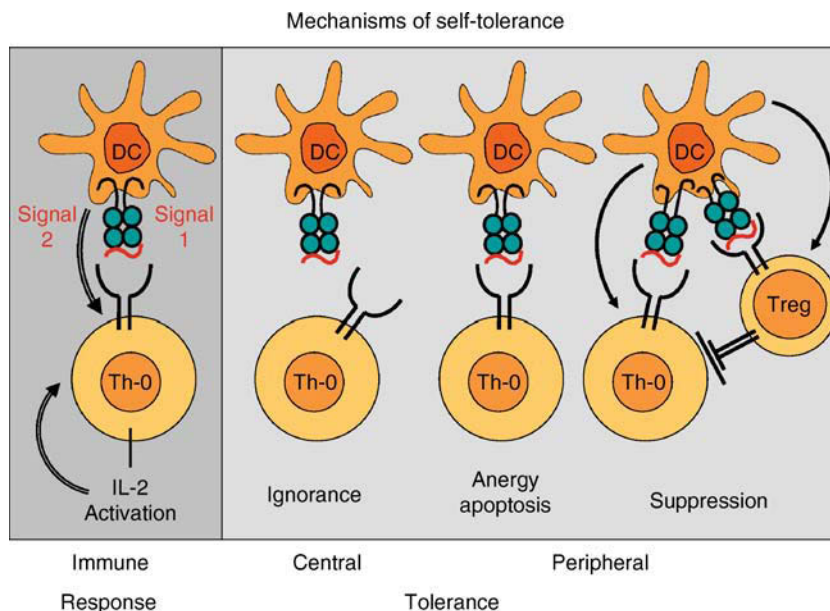
Peripheral self tolerance ensues when the strength of the antigen binding is below a certain threshold, which due to central tolerance holds generally for autoantigens. Also, self antigens elicit only weak signals 2, in sharp contrast to infectious agents. The generation of negative regulating cells (Treg), too, contributes to self tolerance

Breaking Self-Tolerance Leads to Autoimmune Disease

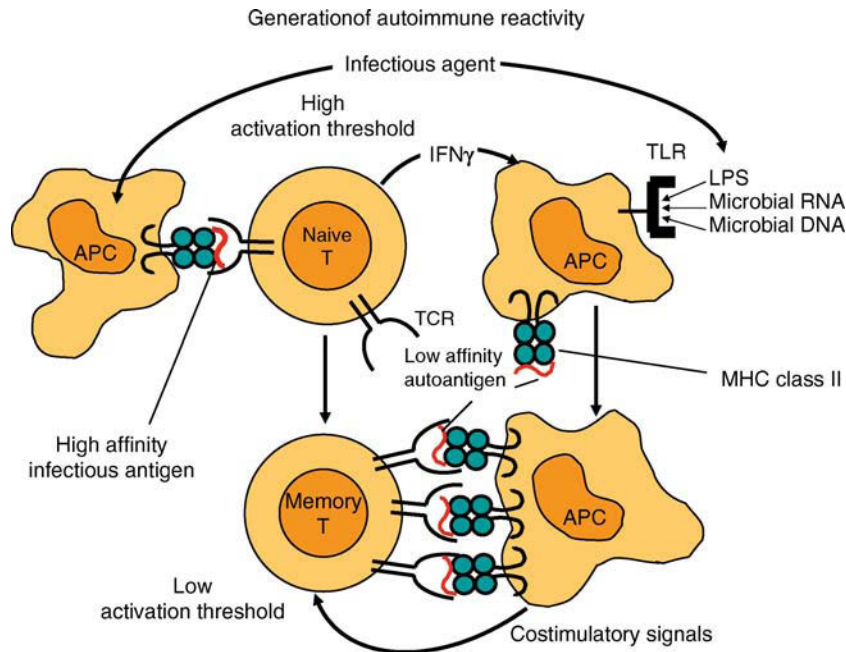
How self-tolerance is broken to result in autoimmune reactions leading to disease is one of the central issues that are still not completely understood. The crucial point is how the reaction barrier in T-lymphocytes is overcome. The trigger must come from the outside; accumulating evidence points to an infection. As T-lymphocytes recognize as their antigen short peptides (below 25 amino acids) one can imagine that proteins of the host as well as of microbes contain short stretches of a similar amino acid sequence. Indeed, numerous examples for this have been documented. Such a related microbial peptide can be bound by a T-cell receptor with an affinity above the threshold for activation. Moreover, other structures of the microbe can effectively generate signal 2 in the antigen-presenting cells. Both together then lead to activation of the T-lymphocytes. These proliferate and also provide sufficient help for the B-lymphocytes to also become activated.

Part of these T-lymphocytes transform into memory cells. These cells are different from their ancestors in that they are activated by a much lower antigen binding strength and also much less depend on signal 2. Now self-antigens can activate these T-lymphocytes. As during activation continuously new memory cells are formed, autoreactivity is sustained and autoimmune disease follows (Fig. 2).

Although this may suffice, additional factors contribute to this process. Activated T-lymphocytes secrete amongst other cytokines also interferon γ which



Autoimmune Disease. Figure 1 Mechanisms of self tolerance. DC, dendritic (antigen presenting) cell; T, T-lymphocyte; Th, T helper lymphocyte; Treg, T regulatory lymphocyte. For details see text.



Autoimmune Disease. Figure 2 Generation of autoreactivity. APC, antigen presenting cell; IFN, interferon; LPS, lipopolysaccharide; MHC, major histocompatibility complex; T, T-lymphocyte; TCR, T cell (antigen) receptor; TLR, toll like receptors. For details see text.

upregulates the expression of MHC molecules and thus improves antigen presentation. The activated antigen presenting cells also enhance the expression and secrete costimulatory molecules.

The activated T- and B-lymphocytes recruit and activate the cells of the innate immune system which mount an inflammatory reaction which impedes and eventually destroys the inflicted organ. On the other hand, by expressing and secreting costimulatory molecules the inflammatory cells also contribute to the activation of T-lymphocytes. Moreover, antigens not accessible to lymphocytes before and (“hidden antigens”) may be exposed, such as cartilage. The local milieu of an inflammation thus not only executes an autoimmune reaction converting it into a disease, but also plays a central role in perpetuating autoimmunity.

Autoimmune Diseases

► **Autoimmune diseases** may inflict on each organ or cell. Manifestations range from affecting a single cell type and its specific function (such as the β -cell of the islands in the pancreas) to systemic diseases which have a detrimental effect on an entire organ system (e.g. the vasculature) of even many different organs. [Table 1](#) summarizes some clinically important diseases.

Also, the outcome covers a large spectrum. Auto-antibodies can specifically block an important protein (such as the gastric intrinsic factor required for the uptake of orally taken vitamin B12), or the receptor for $\rightarrow$ acetylcholine (as in myasthenia gravis), but also can

lead to a continuous stimulation of a receptor (as in autoimmune thyroiditis). Autoimmune reactions can lead to the destruction of a single cell type, the function of which is vital (as in type 1 diabetes (IDDM), where the loss of the β -cells results in the loss of insulin synthesis). In many cases autoimmune disease is associated with chronic inflammation. In fact, most chronic inflammatory diseases are today generally regarded as autoimmune with respect to their mechanisms of perpetuation. This includes rheumatoid arthritis (RA), chronic inflammatory bowel disease (IBD), such as Crohn’s disease or ulcerative colitis, kidney diseases (glomerulonephritis, GN), multiple sclerosis (MS), or arteriosclerosis. Accumulating evidence also points to an autoimmune component in a vast array of diseases up to psychiatric syndromes such as depressive disorders.

Type 1 Diabetes Mellitus (IDDM)

Blood sugar (blood glucose) in human beings is controlled by the secretion of ($\rightarrow$) insulin by the beta (B- or β -) cells of the islands of Langerhans in the pancreas. Loss of insulin synthesis leads to ($\rightarrow$) diabetes. Type 1 diabetes (insulin dependent diabetes mellitus, IDDM) begins in juveniles as an organ-specific autoimmune reaction, the destructive insulinitis.

The cellular infiltrate contains macrophages, T- and B-lymphocytes. Lymphocytes recognize several auto-antigens of the β -cells, including insulin and its precursor proinsulin; Accordingly, auto-antibodies against these antigens are also present. With time the insulinitis leads to a

Autoimmune Disease. Table 1 Autoimmune diseases

Organ-specific autoimmune diseases
Hematologic diseases: autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, pernicious anemia
Kidney disease: Goodpasture syndrom, lipoid nephroses, minimal change glomerulonephritis
Diseases of the gastrointestinal tract: autoimmune chronic active hepatitis, autoimmune atrophic gastritis, Crohn's disease, ulcerative colitis
Neurological diseases: myasthenia gravis, multiple sklerosis, Guillain-Barré-syndrom
Endocrine diseases: autoimmune thyroiditis, primary myxedema, Addison's disease, type 1 diabetes mellitus (IDDM)
Eye diseases: sympathetic ophthalmia, uveitis
Skin diseases: pemphigus, (sklerodermia), psoriasis
Systemic autoimmune diseases
Systemic lupus erythematosus
Rheumatoid arthritis
Mixed connective tissue disease (MCTD)
Sjögren syndrom
Polymyositis
Systemic sklerosis
Systemic vasculitis: Wegener's disease, giant cell arteritis, polyarteritis nodosa

complete and selective loss of the β -cells. What triggers the autoimmune reaction is not known. Epidemiological evidence points to virus infection as infections with coxackie virus B4.

Multiple Sklerosis

Multiple sklerosis is the most frequent neurological disease of jung adults in the western world. It results from a chronic inflammation of the central nervous system which leads to focal demyelinated lesions in brain and spinal cord. Although clinically different forms occur in the onset, it mostly proceeds intermittently, where acute phases alternate with full or partial remissions. The disease inevitably ends in progression leading to increasing neurological defects, which lead do severe physical handicaps and eventually death.

For the pathogenesis of multiple sklerosis, autoimmune T-lymphocytes play a predominant role, which are directed against components of the neural myelin sheath. T-lymphocytes by secreting cytokines such as interferon γ maintain the chronic inflammation which destructs the myelin sheath. Also cytotoxic T-lymphocytes may participate directly. The cause of multiple sklerosis is unknown. Significantly increased antibody titers against several virusses, mostly the measles virus, point to a (latent) virus infection initiating the disease.

Rheumatoid Arthritis

Rheumatoid arthritis represents a chronic inflammatory disease of the joints. About 1% of the population in Germany suffers from this disease. Primarily the synovial membranes of the joints are affected, however, the disease can also reach other organs such as the pleura, pericardium organ and skin blood vessels. The inflamed

synovial membran becomes multicellular bearing infiltrates of predominantly lymphoid cells. The drastically increased synovial fluid (which causes swelling of the joints) contains many inflammatory cells, predominantly granulocytes, macrophages and lymphocytes. The inflammatory process leads to the destruction of the cartilage and eventually to the erosion of the bones. This is augmented by the mostly fibroblastic pannus which can invade the bone. A crucial pathophysiological role is played by the cytokines interleukin-1 and tumor necrosis factor. That rheumatoid arthritis represents an autoimmune disease is supported by many animal models. The putative autoantigens in the human disease are not known.

Systemic Lupus Erythematosus (SLE)

The disease affects predominantly young women. Nearly all of the patients suffer from symptoms such as fatigue, weight loss, and fever and have chronic arthritis. In addition, nearly all organs of the body can be affected to various degrees. Clinically, the severity of the disease can vary within wide ranges. In cases where organs are affected, the disease in former times was lethal without therapy within 10 years in 50% of the patients.

Several immunological abnormalities are found. Most important are pathogenic autoantibodies including antinuclear antibodies (amongst others against nucleoprotein particles or double stranded DNA). The blood plasma contains circulating immunocomplexes which result from an insufficient clearing due to exhaustion of complement. The regulation of the activation of T- and B-lymphocytes often exhibits abnormalities.

Chronic Inflammatory Bowel Disease

Two diseases make up for the majority of all inflammatory bowel diseases: Crohn's disease and ulcerative colitis. Crohn's disease is a chronic recurrent inflammation of all layers of the gut wall. Although it can occur anywhere, the major site of manifestation is the regional ileitis. In contrast, ulcerative colitis is restricted to the colon and the inflammation generally only affects the gut mucosa. In both diseases the inflammatory lesions contain lymphocytes which react with several gut-associated auto-antigens. In the blood also respective autoantibodies are found. To protect the host from infectious agents or toxic substances which are ingested, the gut relies on its barrier function in which its innate immune system plays a central role. At least in Crohn's disease a dysfunction of this system appears to be involved. The defective clearance of the commensal gut bacteria and the subsequent continuous flooding of the gut-associated lymphoid cells with them may lead to a perpetuating immune reaction which supports the chronic inflammation.

Pharmacological Intervention

The treatment of an autoimmune disease very much depends on the nature of the clinical outcome it causes. Although the formation of autoantibodies causes the inactivation of the gastric intrinsic factor, the subsequent shortage of vitamin B12 can be easily overcome by supplying it via an parenteral route. Lifelong immunosuppression (with all its side effects) thus is inappropriate. When, however, as in sympathetic ophthalmia, after damage of the first eye the second eye is endangered, an even drastic immunosuppression is mandatory.

A more differentiated discussion is required regarding type 1 diabetes mellitus. Although injected, now mostly human, insulin can substitute the lost function of the destroyed β -cells, it does it in an unphysiological way, requiring restrictions in the way of life. In addition, concomitant disease may cause severe problems and long-term damages cannot fully be avoided. When the disease begins, theoretically immunosuppression should be able to halt it leaving some β -cells alive to secrete insulin in a regulated manner. Clinical studies with ciclosporin ($\rightarrow$ immunosuppressants) based on such considerations were disappointing. Thus, at least with the immunosuppressive drugs available at present, type 1 diabetes is not an indication for immunosuppressive therapy.

As discussed above, the vast majority of autoimmune diseases is associated with chronic inflammation. The therapy thus rests on two principles:

1. Antiinflammation
2. Immunosuppression

The antiinflammatory drugs include the nonsteroidal antiinflammatory drugs (NSAIDs $\rightarrow$ analgesics) $\rightarrow$ cyclooxygenases), the disease modifying antirheumatic

drugs (DMARDs), such as $\rightarrow$ methotrexate or $\rightarrow$ glucocorticoids. More recently, specific cytokine inhibitors were included which block the action of central mediators of inflammation such as tumor necrosis factor (infliximab, adalimumab, etanercept) or interleukin-1 (anakinra) ($\rightarrow$ cytokines).

Amongst the $\rightarrow$ immunosuppressive agents besides the $\rightarrow$ glucocorticoids the modern nontoxic drugs have become drugs of choice including ciclosporin, tacrolimus or ascomycin.

Paving the way for a new class of drugs effective in autoimmune diseases, immunomodulators have been introduced very recently (mostly to treat rheumatoid arthritis). Abatacept by blocking accessory signals (see Fig. 1) prevents the activation of (autoimmune) T-lymphocytes. Rituximab (which has become a standard drug for treating chronic lymphatic leucemia) by decreasing the number of B-lymphocytes inhibits the formation of autoantibodies. As B-lymphocytes in chronic situation become the predominant antigen presenting cells, the activation of (autoimmune) T-lymphocytes is also impeded. Interferon β ($\rightarrow$ interferons) has become a valuable drug to reduce the number of relapse rates multiple sclerosis. Its major effect appears to be based on tightening the (disease-associated loose) blood brain barrier, and thus preventing autoimmune lymphocytes from entering the central nervous system.

► Interferons

► Immunosuppressive Agents

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Autonomic Nervous System

The part of the vertebrate nervous system that regulates involuntary action, such as the intestines, heart and glands: it is divided into the sympathetic nervous system and the parasympathetic nervous system.

- ▶ α -Adrenergic System
- ▶ β -Adrenergic System
- ▶ Muscarinic Receptors
- ▶ Nicotinic Receptors
- ▶ Purinergic System

Autophagy

Autophagy derived from latin words “self eating” is a normal regulated cell process where cytoplasmic materials are degraded through the lysosomal machinery and the contents reused by the cell. During this process, organelles like mitochondria together with long-lived proteins are sequestered in a double-membrane vesicle delivered and degrade in lysosomes inside the cell. Autophagy is activated in case of nutrient deprivation and plays a crucial role in the destruction of bacteria, viruses, and unnecessary proteins aggregates in cell.

- ▶ Phospholipid Kinases

Autoreactive Lymphocytes

T- or B-lymphocytes which react with autoantigens. In healthy individuals kept under control by the mechanisms of self tolerance.

- ▶ Autoimmune Disease

Autoreceptor

A receptor on nerve endings within a synapse that responds to the released neurotransmitter from that neuron. This then feeds back to the same neuron and negatively regulates the synthesis and release of that neurotransmitter.

- ▶ Synaptic Transmission
- ▶ α -Adrenergic System
- ▶ Muscarinic Receptors
- ▶ Histaminergic System

Autosomal Dominant Hypocalcemia (ADH)

A form of hypoparathyroidism (hypofunction of the parathyroid glands) caused by the presence of activating mutations in the CaR, usually in the heterozygous state.

- ▶ Ca^{2+} -Sensing Receptor

Autotaxin

Autotaxin is a lysophospholipase D that occurs in plasma and serum and cleaves lysophosphatidylcholine, thereby forming lysophosphatidic acid (LPA). This enzyme occurs as a ~125 kDa protein, attached to intracellular vesicles with a single transmembrane domain, and as a soluble extracellular enzyme generated from the former by proteolytic processing and secretion. Autotaxin appears to be a major source of extracellular LPA. In mice expressing only one allele of autotaxin, plasma levels of LPA are half as high as in control mice. Mice with homozygous autotaxin deficiency died around embryonic day 10 with major vascular defects in yolk sac and embryo. They also had allantois malformation, neural tube defects and asymmetric headfolds. These symptoms strongly resemble the phenotype of $\text{G}\alpha_{13}$ knockout mice, suggesting that LPA-GPCR predominantly signal through $\text{G}\alpha_{13}$ in early development.

- ▶ Lysophospholipids

Axon

Long nerve-cell process transmitting the action potential and ending as the synapse.

Axon Reflex

This is an unconventional reflex mediated by capsaicin-sensitive primary afferent neurons. In fact, an adequate stimulus can directly excite a peripheral terminal

generating an action potential. The action potential orthodromically conveys the stimulus to the spinal cord (and eventually triggers a conventional reflex) and/or antidromically invades another peripheral branch of the neurons and induces the release of neuropeptides (and other mediators) at a peripheral site (axon reflex) distal to the site of stimulation.

► **Tachykinins and their Receptors**

primarily repulsive. There is growing evidence that the understanding of the molecular mechanisms of axonal guidance may also provide new approaches for the improvement of regenerative processes after neuronal injury.

► **Plexins**

Axonal Guidance

During the development of the nervous system growing axons find their ways to their final target sites due to the presence of a variety of attractive and repulsive cues in the extracellular environment. These so-called guidance factors act especially on the axonal growth cone which is localized at the tip of the growing axon. Several conserved families of axon guidance factors have been identified. Slits are secreted proteins which repel growth cones by activating Robo (roundabout) class receptors. Netrins can attract or repel axons via their receptors DCC and UNC5. Ephrins are transmembrane proteins that activate Ephs which are receptor tyrosine kinases. The ephrin/Eph system can mediate repulsive as well as attractive signals. Finally, the semaphorins which act mainly via plexins and neuropilins are

Axonal Membrane

The axonal membrane is a lipid bilayer in the nerve fibre. Ionic channels and other proteins are located in the membrane to achieve electrical activity. Action potentials are generated and conducted along the membrane.

► **Local Anaesthetics**

Azole

Antifungal Drugs.

B-Leukotriene

► Leukotrienes

B Lymphocyte

A B lymphocyte is a specific type of white blood cell (leucocyte) derived from bone marrow stem cells. Each B lymphocyte expresses an immunoglobulin (antibody) specific for a particular antigen. Following antigenic stimulation, a B lymphocyte may differentiate and multiply into plasma cells that secrete large quantities of monoclonal antibody.

- Immune Defense
- Humanized Monoclonal Antibodies

BAC

A Bacterial Artificial Chromosome (BAC) is a vector that allows the propagation of larger exogenous DNA fragments, up to several hundred kb. BACs are propagated in recombination-deficient strains of *E. coli*. They are more stable and easier to handle than yeast artificial chromosomes (YACs).

► Transgenic Animal Models

Back Propagation

Back propagation is the propagation of action potentials from the soma distally to dendrites. If the dendrites

are passive cable transmitting distally evoked postsynaptic potentials to the soma, the dendrites do not have to generate action potentials. Direct patch clamp measurements demonstrated the back propagation of action potentials.

► Voltage-dependent Na^+ Channels

Bacterial Toxins

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Definition

Bacterial protein toxins are proteins that are released by the pathogen into the environment. Thereafter, they target eukaryotic cells to damage the membrane to induce pathogenic signaling by acting on membrane receptors or disturb cell signaling and cell function after entry into the cytosol. The three major aims of bacteria that produce toxins are (i) to enter the host organism, (ii) to inhibit the host immune system, and (iii) to produce an appropriate host niche for their own development.

Protein toxins acting intracellularly are often composed of two subunits (A/B model). One subunit is catalytic (A-subunit) and the other is responsible for binding and cell entry (B-subunit). Following binding to an extracellular membrane receptor, the toxins are endocytosed. From the endosomes, the A-subunit is directly (pH dependent) transferred into the cytosol (e.g., diphtheria toxin and anthrax toxin) or the toxin is transported in a retrograde manner via the golgi to the ER (e.g., cholera toxin), where translocation into the cytosol occurs [1].

Mechanism of Action Toxins Modifying Target Proteins

Protein toxins of this type are generally very potent and efficient because they act catalytically. The toxins usually activate or inactivate key eukaryotic proteins

involved in essential cellular functions by covalent modification. One subfamily catalyzes the ADP-ribosylation of target proteins. For unknown reasons, many toxins of this subfamily modify eukaryotic GTPases. Examples are the diphtheria toxin from toxigenic *Corynebacterium diphtheriae* and the *Pseudomonas aeruginosa* exotoxin A, which ADP-ribosylate elongation factor 2 at diphthamide to cause inhibition of protein synthesis (Table 1). Pertussis toxin from *Bordetella pertussis* and cholera toxin from *Vibrio cholerae* act on heterotrimeric G proteins. Pertussis toxin consists of the catalytic subunit S1 and five binding subunits (S2, S3, 2×S4, and S5) with masses of ~11–26 kDa. Cholera toxin consists of a ~28 kDa A-subunit and five B-subunits (~12 kDa). Although pertussis toxin ADP-ribosylates the α -subunits of the G_i subfamily of G proteins (exception G_z) at a cysteine residue, cholera toxin and the related *E. coli* heat labile toxins ADP-ribosylate α -subunits of the G_s subfamily at an arginine residue. Pertussis toxin-induced ADP-ribosylation blocks the interaction of the G protein with heptahelical receptors (GPCR). The ADP-ribosylation of G_s inhibits intrinsic GTPase activity and persistently

activates the G protein. Increase in cellular cAMP, activation of protein kinase A, and subsequent disturbance of cellular electrolyte secretion is suggested to be the cause of cholera toxin-induced diarrhea [1].

Small GTPases of the Rho family are ADP-ribosylated (e.g., at Asn41 of RhoA) and inactivated by C3-like toxins from *Clostridium botulinum*, *Clostridium limosum*, and *Staphylococcus aureus*. These proteins have a molecular mass of 23–30 kDa and consist only of the enzyme domain. Specific inhibition of Rho functions (Rho but not Rac or Cdc42 are targets) is the reason why C3 is widely used as a pharmacological tool [2].

Another subfamily of ADP-ribosylating toxins modifies G-actin (at Arg177), thereby inhibiting actin polymerization. Members of this family are, for example, *C. botulinum* C2 toxin and *Clostridium perfringens* iota toxin. These toxins are binary in structure. They consist of an enzyme component and a separate binding component, which is structurally related to the binding component of anthrax toxin [3].

The above mentioned Rho GTPases are glucosylated by the family of clostridial glucosylating cytotoxins.

Bacterial Toxins. Table 1 Intracellular acting exotoxins

Toxin	Protein substrate	Activity	Functional consequences
Diphtheria toxin, <i>Pseudomonas</i> exotoxin A	Elongation factor 2	ADP-ribosylation	Inhibition of protein synthesis (diphtheria, <i>Pseudomonas</i> infection)
Cholera toxin, heat labile <i>E. coli</i> toxins	G_s proteins	ADP-ribosylation	Activation of adenylate cyclase (cholera, "traveler"-diarrhea)
Pertussis toxin	$G_{i,o}$ proteins	ADP-ribosylation	Inhibition of G protein signaling (whooping cough)
<i>C. botulinum</i> C2-toxin and related toxins	Actin	ADP-ribosylation	Inhibition of actin polymerization
<i>C. botulinum</i> C3-toxin and related toxins	Rho proteins	ADP-ribosylation	Inhibition of RhoA, B,C Destruction of the cytoskeleton
<i>E. coli</i> CNF1 and 2, <i>Yersinia</i> CNFy	Rho proteins	Deamidation	Activation of RhoA, (Rac, Cdc42)
<i>Bordetella</i> DNT	Rho proteins	Transglutamination	Activation of RhoA, Rac, Cdc42
<i>C. difficile</i> toxin A and B	Rho proteins	Glucosylation	Inactivation of Rho proteins Destruction of the cytoskeleton
Botulinum neurotoxins (A–G), tetanus toxin	Synaptic peptides: a) Synaptobrevin b) Syntaxin c) SNAP25	Zinc dependent endoprotease	Cleavage of synaptic peptides Inhibition of transmitter release (tetanus, botulism)
Shiga toxins and related toxins from <i>E. coli</i>	No proteins (!) 28S rRNA	N-glycosidase	Cleavage of 28S rRNA Inhibition of protein synthesis
Anthrax toxin Lethal factor Lethal factor	MEKs	Endoprotease Increase in intracellular cAMP	Inhibition of MAP-kinase pathways Calmodulin dependent adenylylcyclase

Important members of this toxin family are *Clostridium difficile* toxins A and B, which are implicated in antibiotics-associated diarrhea and pseudomembranous colitis. The large clostridial cytotoxins are single-chain toxins with molecular masses of 250–308 kDa. The enzyme domain is located at the N terminus. The toxins are taken up from an acidic endosomal compartment. They glucosylate RhoA at Thr37; also, Rac and Cdc42 are substrates. Other members of this toxin family such as *Clostridium sordellii* lethal toxin possess a different substrate specificity and modify Rac but not Rho. In addition, Ras subfamily proteins (e.g., Ras, Ral, and Rap) are modified. As for C3, they are widely used as tools to study Rho functions [2] [4].

Rho GTPases are activated by cytotoxic necrotizing factors 1 and 2 (CNF1, 2) from *Escherichia coli*, CNFy from *Yersinia pseudotuberculosis*, and by the dermonecrotic toxin (DNT) from *Bordetella*. CNF1 and CNF2 are more than 90% identical, whereas CNFy has ~60% sequence identity with CNF1. All three toxins are single-chain proteins with molecular masses of about 115 kDa. Their cell-binding domains are located at the N terminus and the catalytic domain at the C terminus of the toxins. DNT is a protein of ~160 kDa that shares significant homology with CNFs in the catalytic domain. All these toxins activate the small GTP-binding proteins of the Rho family by deamidation (CNFs) and transglutamination (DNT) of a glutamine residue (e.g., Gln63 of RhoA), which is necessary for GTP hydrolysis. Moreover, the inactivation of Rho GTPases by GTPase-activating protein (GAP) is blocked by CNFs and DNT. According to the functions of Rho GTPases, the toxins cause formation of stress fibers, filopodia, and membrane ruffles, and induce cell flattening and multinucleation. The role of CNFs in the pathogenesis of *E. coli* infections is still unclear. CNFs and DNT are dermonecrotic after intradermal application [5].

The anthrax toxin is a tripartite toxin and consists of the binding component protective antigen (PA), the lethal factor (LF), which is a metalloprotease, and the edema factor (EF), which is a calmodulin-dependent adenylcyclase. Both enzyme components are translocated via PA into target cells. PA is activated by furin-induced cleavage and forms heptamers, which are similar to the binding components of C2 toxin and iota toxin. In the low pH compartment of endosomes, the heptamers form pores to allow translocation of LF and EF. LF cleaves six of the seven MEKs (MAPK-kinases) thereby inhibiting these enzymes. The functional consequence is the blockade of the MAPK pathways that control cell proliferation, differentiation, inflammation, stress response, and survival. Whether this is the reason for the LT-induced cell death of macrophages is not clear [1].

Shiga toxin is produced (i) by *Shigella dysenterica*, the cause of bacillary dysentery, (ii) by certain *E. coli* strains (EHEC, enterohaemorrhagic *E. coli*; cause of the

hemolytic uremic syndrome, HUS), and (iii) by various enterobacteriaceae (e.g., *Enterobacter cloacae*). The toxin consists of an A-subunit (~32 kDa) and a pentameric B-subunit (7.7 kDa each). The toxin enters cells after retrograde transport to the Golgi. In the cytosol, the A-subunit acts as an N-glycosidase to remove one adenine residue in position 4324 of the 28S rRNA at the ribosome and blocks protein biosynthesis [1].

Clostridial neurotoxins are mainly bichain toxins having a ~50 kDa enzyme component and a ~100 kDa binding/translocation subunit. They are the cause of botulism, a generalized flaccid paralysis of skeletal muscles, mainly acquired by foodborne poisoning, and tetanus, which occurs subsequent to wound infection. Botulism is induced by *C. botulinum* neurotoxins types A, B, C1, D, E, F, and G. Tetanus is induced by tetanus toxin from *Clostridium tetani*. The toxins belong to the most potent agents known. About 1 ng of botulinum neurotoxin per kg body mass may be lethal for man or animal. The toxins are zinc metalloproteases and cleave synaptic peptides involved in transmitter release. Botulinum neurotoxins B, D, F, and G and tetanus toxin cleave synaptobrevin; neurotoxins A and E cleave SNAP25, and neurotoxin C cleaves syntaxin. The botulinum neurotoxins induce flaccid muscle paralysis (botulism) because they act presynaptically at the peripheral neuromuscular junction to block acetylcholine release. Tetanus toxin is taken up at the neuromuscular junction but is then transported in a retrograde manner to the spinal cord. Within the spinal cord, tetanus toxin migrates to interneurons and blocks the release of inhibitory transmitters to cause spastic paralysis [1].

Pasteurella multocida toxin (PMT) is the major pathogenic factor responsible for atrophic rhinitis, a disease which is characterized by bone loss in the nose of pigs. PMT is a 145 kDa single-chain exotoxin, which activates $G\alpha_q$ protein (but not $G\alpha_{11}$) and stimulates phospholipase C β . In addition, $G_{12/13}$ proteins and subsequently Rho pathways are activated.

Bacterial Phospholipases

The α -toxin from *C. perfringens* is involved in the pathogenesis of gas gangrene and the sudden death syndrome of young animals. This toxin is a zinc metalloenzyme with phospholipase activity. Other phospholipase C-toxins are from *Listeria monocytogenes* and *Mycobacterium tuberculosis*. The phospholipase C-toxin from *Bacillus cereus* is specific for phosphatidylinositol. It cleaves phosphatidylinositol and its glucosyl derivatives. In cell biology, this toxin can be used as a tool to study whether a protein is anchored to GPI. A second phospholipase C-toxin produced by *B. cereus* is specific for sphingomyelin. Cleavage of sphingomyelin generates ceramide, a second messenger involved in processes like apoptosis and differentiation.

Pore-Forming Toxins

Pore-forming toxins act by punching holes into mammalian cell membranes. Many different types are known. They can be divided into small and large pore forming toxins. Pore-forming toxins oligomerize in the plasma membrane of the mammalian cell to build circular structures. These ring-like structures can be composed of a few molecules, generating small pores that allow the exchange of ions and nucleotides (*Aeromonas aerolysin*, *S. aureus* α -toxin). Large pores, which allow the passage of peptides or proteins are formed by toxins which insert up to 50 molecules into the plasma membrane generating a pore with up to 35 nm diameter. Examples for such toxins are *Streptococcus pyogenes* streptolysin O or *C. tetani* tetanolysin.

Injected toxins are directly delivered into the cytosol of eukaryotic target cells by the bacterial type III secretion system. The pathogens (e.g., *P. aeruginosa*, *Yersinia*, and *Salmonella*) produce a set of proteins that are delivered into mammalian cells by this complex type III secretion machinery depending on the direct contact between bacterium and host cells. Some of these injected toxins (e.g., Yops in the case of *Yersinia*) do not covalently modify mammalian targets but act as modulators on important signal transduction pathways; they act as molecular mimics of cellular proteins. For example, they regulate the activity of small GTP-binding proteins as exchange factors to activate the small G proteins or as activators of GTP-hydrolysis to inhibit them. Notably, *Salmonella* produces two contrary acting molecular mimics. They inject an activator of Rho GTPases (SopE) to induce ruffling and the uptake of the bacteria into the mammalian cell, and they inject an inactivator of Rho GTPases (SptP) probably to switch off the induced cytoskeletal rearrangements.

Superantigens

Like physiological ligands, bacterial toxins can influence cells by binding to cell surface molecules. Best known are the superantigens produced by *Staphylococcus* strains. Superantigens are bivalent molecules that bind to the major histocompatibility complex (MHC) class II and to the variable regions of the T-cell receptor. This bridging leads to the activation of the T-cell receptor in the absence of an antigenic peptide. This unspecific activation of T-cells is followed by a massive release of cytokines which is thought to play a role in diseases like toxic shock syndrome and some exanthemas.

Clinical Uses

Botulinum neurotoxins are widely used as therapeutic agents to cause reduction or paralysis of skeletal muscle contraction. They are used to treat cervical dystonia, which causes regional involuntary muscle spasms often associated with pain. Moreover, they are used in strabism, blepharospasm, hemifacial spasm, and

achalasia. Meanwhile, a number of studies indicate efficacy for botulinum toxin for the treatment of tension headache, but further studies are necessary to demonstrate its short-term and long-term efficacy. Botulinum toxin is also used as a cosmetic agent and the effects occur a few days after injection into the muscle and last for several months. The treatment can be repeated several times without major development of antineurotoxin antibodies. In the case of antineurotoxin antibody production, the treatment is continued with a different botulinum neurotoxin.

Some toxins (e.g., the diphtheria toxin) fused to antibodies are used as immunotoxins against cell surface molecules, for example, to deplete T-cells as targeted therapy for cutaneous T-cell lymphoma. The use of bacteria with the type III secretion system (e.g., *Salmonella*) producing only an injected toxin-antigen fusion protein as live vaccines is under current investigation. The aim is to directly deliver protein fragments into antigen-presenting cells to improve immunization.

- Cytoskeleton
- Exocytosis
- Small GTPases

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Bacteriophage

A bacteriophage (or phage) is a virus, made up of a DNA or RNA core and a protein coat that may infect bacteria.

- Humanized Monoclonal Antibodies

Barbiturates

Barbiturates are salts of barbituric acid. They can be used as hypnotics, sedatives, general anaesthetics, and

antiepileptics. Similar to benzodiazepines they have modulatory effects on the GABA_A receptor.

► GABAergic System

Baroreceptor Reflex

The baroreceptor reflex is a central reflex mechanism, which reduces heart rate following an increase in blood pressure. Each change in blood pressure is sensed by baroreceptors in the carotid arteries, which activate the autonomic nervous system to alter heart rate and thereby readjust blood pressure.

- Blood Pressure Control
- Renin–Angiotensin–Aldosterone System

β Barrel

A beta barrel is a three-dimensional protein fold motif in which beta strands connected by loops form a barrel-like structure. For example, this fold motif is found in many proteins of the immunoglobulin family and of the chymotrypsin family of serine proteases.

Bartter's Syndrome

Bartter's syndrome (antenatal Bartter syndrome, hyperprostaglandin E syndrome) is an autosomal-recessive electrolyte disorder, producing hypokalemia, metabolic alkalosis, hyper-reninism, and hyperaldosteronisms. It has now been recognized to be caused by mutations in at least three transport proteins responsible for NaCl absorption in the loop of Henle. Besides mutations in the Na⁺/K⁺/2Cl-cotransporter, Bartter's syndrome can also be caused by mutations in the K⁺ channel that is present in the apical membrane of the ascending limb (ROMK or KIR1.1). This K channel is a K-recycling pathway and its operation is a prerequisite for NaCl absorption through NKCC2. Clinically, Bartter syndromes types I and II are indistinguishable. In contrast, a milder form of

Bartter's syndrome is caused by mutations in the basolateral chloride channel (ClC-Kb), an exit pathway for cellular Cl.

- Diuretics
- K⁺ Channels

Basal Activity

Basal activity is the enzymatic or other activity of a protein normally governed by interaction with a ligand or other activator molecule in the absence of that activator.

Basal Ganglia

Basal ganglia are a group of subcortical nuclei which are essential for the coordination of movements (so-called extrapyramidal system). They include the caudate nucleus, putamen, globus pallidus, and lenticular nucleus. Damage of the basal ganglia results in involuntary movements, as are observed in Parkinson's disease and Huntington's chorea.

- Anti-Parkinson Drugs

Basement Membrane

The basement membrane is a structure that supports overlying epithelial or endothelial cells. The primary function of the basement membrane is to anchor down the epithelium to its loose connective tissue underneath. This is achieved by cell–matrix adhesions through cell adhesion molecules.

- Matrix Metalloproteinases

Basophils

Basophils constitute a subgroup of circulating blood cells (leucocytes). In many aspects they resemble

non-circulating mast cells. Upon binding of antibody of the IgE class, basophils release histamine and other proinflammatory agents.

► Allergy

Bax

Bax is a bcl-2 homolog that forms with bcl-2 and acts to accelerate apoptosis.

- Apoptosis
- Neurodegeneration

Bazedoxifene

Bazedoxifene is a third generation SERM that displays estrogenic effects in bone and the cardiovascular system, but functions as an antiestrogen in the breast and uterus.

- Selective Sex Steroid Receptor Modulators

Bcl-2

Bcl-2 (B-cell lymphoma-related gene) is major mammalian gene that is known to inhibit apoptosis.

- Apoptosis
- Neurodegeneration

Bcl-x

Bcl-x is a gene in the bcl-2 family that inhibits apoptosis after trophic factor deprivation in vitro.

- Apoptosis
- Neurodegeneration

BCR-ABL Fusion Protein

The discovery of the Philadelphia chromosome (in Philadelphia in 1960) led to the identification in chronic myeloid leukemia (CML) cells of the BCR-ABL fusion gene and its corresponding protein. ABL and BCR encoding genes are normally located on chromosomes 9 and 22, respectively. The ABL gene encodes a tyrosine kinase enzyme whose activity is tightly regulated. By the Philadelphia translocation, two fusion genes are generated: BCR-ABL on the Philadelphia chromosome (abbreviated chromosome 2Z) and ABL-BCR on the chromosome 9. The BCR-ABL gene encodes a protein with deregulated tyrosine kinase activity. The presence of this protein in the CML cells is strong evidence of its pathogenetic role. The efficacy in CML of a drug that inhibits the BCR-ABL tyrosine kinase has provided the final proof that the BCR-ABL oncoprotein is the unique cause of CML.

- Tyrosine Kinases
- Targeted Cancer Therapy

BCRP

The breast cancer resistance protein (BCRP) belongs to the G-branch of the ABC-transporter family (ABCG2). In contrast to most other ABC-proteins, BCRP consists of only one transmembrane domain (TMD) with one nucleotide binding fold (NBF) at its C-terminus. Because of this structural characteristic BCRP as well as other ABC-transporters with only one TMD are termed half transporters. To achieve functional activity these transporters have to form hetero- or homodimers. BCRP is involved in the multidrug resistance of certain tumors and transports endogenous compounds like cholesterol and steroid hormones.

- ABC-Transporter
- MDR-ABC Transporters

BDNF

BDNF (brain-derived neurotrophic factor) is a neurotrophin, i.e. a target-derived growth factor, which is expressed in the brain predominantly in the hippocampus. It acts through its tyrosine kinase receptor, trkB,

which after ligand activation induces phosphorylation of intracellular signalling proteins on tyrosine residues. BDNF expression is suppressed by stress hormones, and increased by antidepressants through a yet unknown mechanism.

- Neurotrophic Factors
- Pain and Nociception
- Antidepressant Drugs

Behavioral State

Levels of arousal (sleep–awake), vigilance (attentive–distracted), mood (appetitive–aversive), and movement (flexible–immobile) of an organism at a given time point or context.

- Sleep
- Orexins
- Psychostimulants

Benign Familial Neonatal Convulsions

Benign familial neonatal convulsion is an idiopathic form of epilepsy beginning within the first six months after birth. Seizures include generalized and mixed, starting with tonic posture, ocular symptoms, and apnea, and often progress to clonic movements and motor automatisms.

- K^+ Channels
- Antiepileptic Drugs

Benzo(a)pyrene

One of the most studied of the polycyclic aromatic hydrocarbons (PAH) is benzo(a)pyrene (BaP), which is present in coal tar at coke oven plants. The BaP content of coal tar is between 0.1% and 1% and it contributes to the serious potential health effects on employees exposed to coke oven emissions. The largest sources of BaP are open burning and home heating with wood and coal. The latter alone contributes 40 percent of all the BaP released each year in the USA.

Industries that burn wood, gas, oil or coal contribute most of the rest of airborne B(a)P. Studies on animals have shown that contact with BaP and PAH can cause skin cancer, but the effects of breathing or ingesting them are not yet well enough studied to draw a conclusion as to other cancers. Animal tests have shown that exposure to BaP may cause reproduction difficulty. The U.S. government considers BaP a human carcinogen.

Benzodiazepine Receptor Agonists

Synonyms

BzRAs

Definition

A class of sedative/hypnotic type drug that exert their effects through the benzodiazepine binding site on GABA_A receptors. The class consists both of molecules that contain the benzodiazepine moiety, for example diazepam, lorazepam and flunitrazepam, and the newer, non-benzodiazepine compounds such as zolpidem, zopiclone, indiplon and zaleplon. BzRAs are primarily used for the treatment of anxiety, insomnia and to elicit varying levels of sedation. The wide selection of compounds currently available affords the prescribing clinician extensive options in terms of relative efficacies and durations of action.

- Sleep
- Benzodiazepines

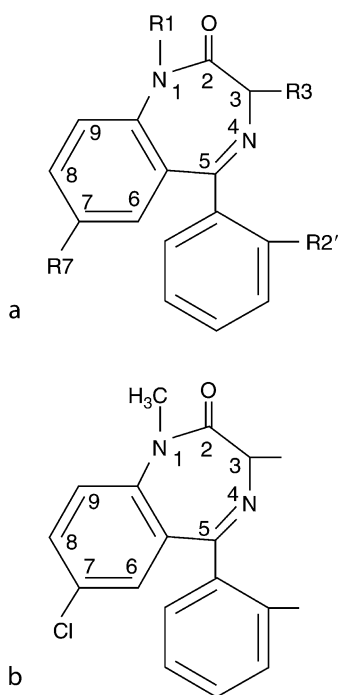
Benzodiazepines

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Definition

The term benzodiazepine refers to a chemical structure, consisting of a heterocyclic ring system in which the two N atoms are mostly located in positions 1 and 4 (1,4-benzodiazepines), e.g., in diazepam (Fig. 1).

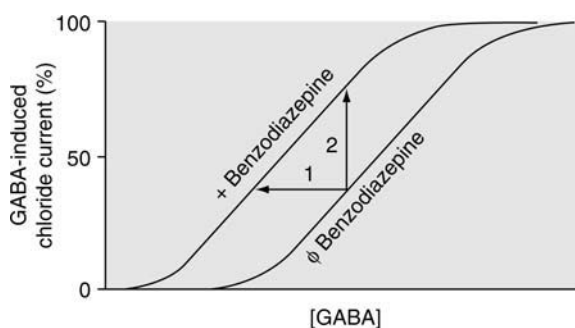


Benzodiazepines. Figure 1 (A) Chemical structure of classical [1,4] benzodiazepines. R1, R2, R2', R3, and R7 denote variable substituents. (B) Chemical structure of the prototypical benzodiazepine diazepam.

Benzodiazepines have found wide therapeutic applications as ►anxiolytics, sedatives, hypnotics, anticonvulsants, and central muscle relaxants.

Mechanism of Action Enhancement of GABA Response

►GABA is the major inhibitory neurotransmitter in the mammalian central nervous system. Its fast synaptic actions are mediated by ►GABA_A receptors, which are located on postsynaptic membranes. GABA_A receptors have a central pore, with selectivity for chloride ions. Upon binding of GABA to GABA_A receptors, negatively charged chloride ions flow into the postsynaptic neuron, leading – in most cases – to hyperpolarization of the postsynaptic membrane and thus functional inhibition. In addition to a binding site for the physiological neurotransmitter GABA, most GABA_A receptors contain binding sites for ►allosteric modulators, e.g., benzodiazepines, ►barbiturates, and ►neurosteroids. Benzodiazepines bind to a common modulatory site that is called benzodiazepine site. However, the ligands of the benzodiazepine site are not limited to ligands of the benzodiazepine structure. In particular, the imidazopyridine zolpidem, a widely used hypnotic, and zopiclone, a cyclopyrrolone, also bind to the benzodiazepine site. The basic mechanism of action of benzodiazepines and nonbenzodiazepines acting via the benzodiazepine site appears to be the same.



Benzodiazepines. Figure 2 Schematic representation of the potentiation of the GABA-induced chloride current by benzodiazepines. The GABA dose–response curve is shifted to the left in the presence of benzodiazepines (arrow 1). The chloride current that is induced by a submaximal concentration of GABA is increased (arrow 2). Benzodiazepines are effective only in the presence of GABA (use-dependence) and cannot increase the inhibition by high concentrations of GABA (self-limiting action).

The binding of a benzodiazepine to the benzodiazepine site of the GABA_A receptor enhances GABAergic inhibition by increasing the opening frequency of the GABA-gated ion channel. This leads to a shift of the GABA dose–response curve to the left, so that at any given concentration of GABA, the response is increased (Fig. 2). This can also be viewed as an increase in the affinity of GABA for the receptor. The action of benzodiazepines is use-dependent, and self-limiting. Use-dependence indicates that benzodiazepines are only active in the presence of GABA. In the absence of GABA, benzodiazepines do not have an effect on their own, i.e., their action is dependent on the precondition that GABA is present, and the respective synapse thus in use. Furthermore, benzodiazepines are not able to increase the response to GABA beyond its physiological maximum at high GABA concentrations, which is referred to as the self-limiting nature of their action. The magnitude of the effect of benzodiazepines depends on the amount of GABA present in the synapse and hence, synaptic activity. The self-limiting feature may help explain why the enhancement of GABA transmission by benzodiazepines is typically safe even at high doses, whereas overdoses with drugs that do not display this self-limiting feature, e.g., barbiturates, are life-threatening.

Subtype-Specificity of BZ Actions

GABA_A receptors that contain the $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits in combination with β and γ subunits can bind classical benzodiazepines, e.g., diazepam, whereas GABA_A receptors that contain the $\alpha 4$ and $\alpha 6$ subunits do not bind classical benzodiazepines. Essentially, all benzodiazepines that are currently in clinical use bind indiscriminately to GABA_A receptors that contain the

$\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits. The only clinically used drug that displays a significant subtype selectivity is the imidazopyridine hypnotic, zolpidem. Zolpidem has a high affinity at GABA_A receptors containing the $\alpha 1$ subunit, an intermediate affinity at GABA_A receptors containing the $\alpha 2$ or $\alpha 3$ subunits, and no affinity at GABA_A receptors containing the $\alpha 5$ subunit. The GABA_A receptor subtype-specificity of benzodiazepine actions was assessed in genetically engineered mice. Whereas the diazepam-sensitive $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ subunits have a histidine residue in a conserved position in the N-terminal extracellular domain (H101 in $\alpha 1$, H101 in $\alpha 2$, H126 in $\alpha 3$, H105 in $\alpha 5$), the diazepam-insensitive $\alpha 4$ and $\alpha 6$ subunits have an arginine residue at the homologous position. By mutating the conserved histidine residue in the $\alpha 1$, $\alpha 3$, and $\alpha 5$ subunits to arginine residues, the GABA_A receptors containing the respective subunits were rendered diazepam-insensitive. Using this approach, it was discovered that the sedative and anterograde amnesic action of diazepam, and in part also the anticonvulsant action of diazepam, are mediated by GABA_A receptors containing the $\alpha 1$ subunits, while the anxiolytic action of diazepam is mediated by GABA_A receptors containing the $\alpha 2$ subunit. The central muscle relaxant action of diazepam is mediated by GABA_A receptors containing the $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits (Fig. 3) [1–5]. The anxiolytic action of diazepam is observed at much lower doses than the muscle relaxant action. Interestingly, GABA_A receptors, containing the $\alpha 3$ subunit were not involved in mediating the anxiolytic-like action of diazepam in ethological tests of anxiety, indicating that this response is not dependent on neurons in the reticular activating system, where the $\alpha 3$ subunit is expressed.

These findings demonstrate that subtype-selective drugs are likely to be of benefit, e.g., as anxiolytics

without sedative and anterograde amnesic side effects. A remarkable step in this direction is the development of L-838,417, which is a partial agonist at GABA_A receptors, containing the $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits, but has no activity at GABA_A receptors, containing the $\alpha 1$ subunit. This compound is active as an anxiolytic and anticonvulsant, but apparently does not impair motor performance [4].

Interestingly, while the sedative action of diazepam is mediated by GABA_A receptors containing the $\alpha 1$ subunit, its **▶REM** sleep inhibiting action, its enhancement of sleep continuity, and its effect on the **▶EEG** spectra in sleep and waking, are mediated by GABA_A receptors that do not contain the $\alpha 1$ subunit, indicating that the hypnotic effect of diazepam and its EEG fingerprint can be dissociated from its sedative action [6].

Agonists and Inverse Agonists

Drugs that bind to the benzodiazepine site of the GABA_A receptor and enhance GABA responses, are termed agonists. Essentially all ligands at the benzodiazepine site that are in clinical use are agonists. In contrast, inverse agonists diminish GABA responses. They are not in clinical use, and have effects opposite to those of the agonists, e.g., they are convulsant and anxiogenic.

The Antagonist Flumazenil

Although flumazenil binds with high affinity to the benzodiazepine site of GABA_A receptors, it has practically no action when given alone. However, flumazenil competitively blocks the action of benzodiazepine site agonists. Flumazenil can be used to terminate the action of benzodiazepines, e.g., after a benzodiazepine overdose. It may also serve as a diagnostic tool in this regard.

Pharmacokinetic Considerations

The benzodiazepines, currently on the market differ in their pharmacokinetic properties, in particular, the duration of action, which guides the use of the drug to be used. The half-life is largely determined by the rate of metabolic degradation of the parent drug. In addition, long-acting metabolites (e.g. desmethyldiazepam) are generated that may contribute to the duration of action. Short-acting drugs might be used for patients with difficulties to fall asleep, with the expectation that there is no hangover effect on the next day. Long-acting drugs may be used if reawakening during the entire night is to be prevented. Short-acting benzodiazepines may have a half-life in the range of 2–6 h, e.g., midazolam, triazolam, and oxazepam, medium-acting benzodiazepines with a half-life in the range of 10–12 h, e.g., lorazepam and lormetazepam, and long-acting benzodiazepines with a half-life in the range of 20–50 h, e.g., diazepam and clobazam.

	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 5$
Sedation	+	–	–	–
Anterograde amnesia	+	ND	ND	ND
Anticonvulsant activity	+	–	–	–
Anxiolysis	–	+	–	–
Myorelaxation	–	+	+	+

Benzodiazepines. Figure 3 Dissection of benzodiazepine pharmacology. The functional roles of GABA_A receptor subtypes, mediating particular actions of diazepam, are indicated. A “+” sign indicates that the respective response is mediated by the respective receptor subtype, a “–” sign indicates that the respective response is apparently not mediated by the respective receptor subtype. ND = not determined.

Clinical Use

Benzodiazepines are amongst the most frequently prescribed drugs; they have well-established uses in the treatment of anxiety disorders (anxiolytics) and insomnia, preanaesthetic sedation, suppression of seizures, and muscle relaxation.

Benzodiazepines are used as tranquilizers to relieve anxiety states, e.g., in generalized anxiety disorder and panic attacks. The anxiolytic effects are observed at low doses, suggesting that only a small number of GABA_A receptors need to be modulated to obtain the anxiolytic effect. As outlined previously, this action is, most likely, mediated by GABA_A receptors containing the $\alpha 2$ subunit. In contrast, higher doses of benzodiazepines and thus a higher receptor occupancy is needed for the sedative action of diazepam, which is mediated by GABA_A receptors containing the $\alpha 1$ subunit. When diazepam is used as an anxiolytic, sedative side effects are frequently troublesome. The reduction of the reactivity to external stimuli is the basis for the use of benzodiazepines as hypnotics in the treatment of sleep disorders. The anticonvulsant activity of diazepam can be explained by the GABAergic inhibition of neuronal responsiveness to excitatory inputs. Benzodiazepines (lorazepam and diazepam) are the drugs of choice in the treatment of status epilepticus. Their use in the chronic treatment of epilepsy (e.g. clonazepam) is limited by the development of ►tolerance.

The definition of desired therapeutic and side effects in the case of the benzodiazepines very much depends on the clinical problem in question. The sedative and hypnotic actions are desired effects in the treatment of insomnia, but undesired effects in the treatment of anxiety disorders. Effects that are usually undesired include daytime drowsiness, potentiation of the sedative effects of ethanol, and anterograde amnesia. They are mediated via the benzodiazepine site of GABA_A receptors, since they can be antagonized with flumazenil.

Repeated administration may lead to the development of tolerance to certain benzodiazepine effects, in particular, to the sedative, anticonvulsant and muscle relaxant effects, and to the development of ►physical dependence, which can include withdrawal anxiety, insomnia, convulsions, and sensory hyperactivity and thus being similar to the symptoms that lead to the treatment. To avoid ►withdrawal symptoms, chronic treatment is discontinued by gradually tapering out the dose over a long period of time. The neurobiological nature of the adaptive changes, which occur after long-term treatment or withdrawal from long-term treatment, are poorly understood. Because of the adaptive changes that occur under chronic treatment, the long-term use of benzodiazepines is generally not recommended. For treatment of insomnia, benzodiazepines should not be given for more than e.g., 4 weeks. For the treatment of

anxiety disorders, benzodiazepines should not be used for more than e.g., 6 months. Because of their potentiation of the sedative action of ethanol, benzodiazepines should not be used in patients with alcohol abuse. Likewise, the potential nontherapeutic use of benzodiazepines, for the purpose of euphoria, has to be kept in mind, and particular care should be taken while treating patients with a history of drug abuse.

►GABAergic System

►Sleep

►Antiepileptic Drugs

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Benzothiazepines

Benzothiazepines (e.g. Diltiazem) block dihydropyridine-sensitive HVA calcium channels.

►Ca²⁺ Channel Blockers

►Voltage-dependent Ca²⁺ Channels

Benzoylcholinesterase

►Cholinesterases

Beri-Beri

Beri-beri or clinically manifest thiamin deficiency exists in several subforms: infantile beri-beri and adult beri-beri. Infantile beri-beri occurs in exclusively breastfed infants of thiamin-deficient mothers. Adults can develop different forms of the disease, depending on their constitution, environmental conditions, the relative contribution of other nutrients to the diet as well as the duration and severity of deficiency. First of all, there is a so called dry or atrophic (paralytic or nervous) form, including peripheral degenerative polyneuropathy, muscle weakness and paralysis. Second, a wet or exudative (cardiac) form exists. In this form, typical symptoms are lung and peripheral oedema as well as ascites. Finally, there is a cerebral form, that can occur as Wernicke encephalopathy or Korsakoff psychosis. This latter form mostly affects chronic alcoholics with severe thiamin deficiency.

► Vitamin B1

BH Domain

BH domains or Bcr (breakpoint cluster region) homology domains are homologous to the GTPase activating protein domain of the Bcr gene product. Although the p85 BH domain specifically interacts with the Rho family proteins Cdc42 and Rac1, no GTPase-activating activity has been attributed to it.

► Phospholipid Kinases

Biased Agonism

This term indicates a receptor ligand having different intrinsic activities at different transduction pathways coupled to a single receptor. To quote Urban et al. (2006 JPET, #104463 PiP) and Kenakin (2007 Trends Pharmacol Sci 28:359–361) the term “biased agonism” can be considered as a synonym of “collateral efficacy,”

“functional selectivity,” “agonist-directed trafficking of receptor stimulus,” “protean agonism,” “differential engagement,” or “stimulus trafficking.”

► Tachykinins and their Receptors

Bicuculline

Bicuculline is a competitive antagonist at the GABA_A receptor. It is a plant alkaloid.

► GABA_A Receptor

BID

BID is a member of the Bcl-2 gene family, which encode proteins that function either to promote apoptosis or to inhibit apoptosis as in the proteins derived from Bcl-2. These proteins can exist as monomers or they can dimerize. For example, if two promoting Bcl-2 family proteins dimerize then apoptosis will be greatly enhanced. Conversely, if dimerization of an inhibitory and promotor protein occurs, then the effects are cancelled out. The Bcl-2 family of proteins are localized to the outer mitochondrial or outer nuclear membranes.

► Apoptosis

Biguanide

A class of drug derived from guanidine, including metformin and phenformin. Metformin is currently widely used in humans for the treatment of type 2 diabetes. Phenformin was formerly also widely used but was withdrawn because of problems with lactic acidosis.

► AMP-activated Protein Kinase

► Diabetes Mellitus

► Antidiabetic Drugs other than Insulin

Bile Acids

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Synonyms

Bile salts

Definition

Bile acids are a group of molecules synthesized from cholesterol, containing a sterol nucleus, a variable number of hydroxyl groups and a side chain with a carboxyl group that can be conjugated to taurine or glycine. Their ►**amphipathic** nature is essential to solubilize dietary lipids and fat-soluble vitamins, which subsequently promotes absorption of these molecules in the digestive tract. Besides their classic roles in dietary lipid absorption and cholesterol homeostasis, it is now evident that bile acids are also signaling molecules that play important roles in metabolic ►**homeostasis**.

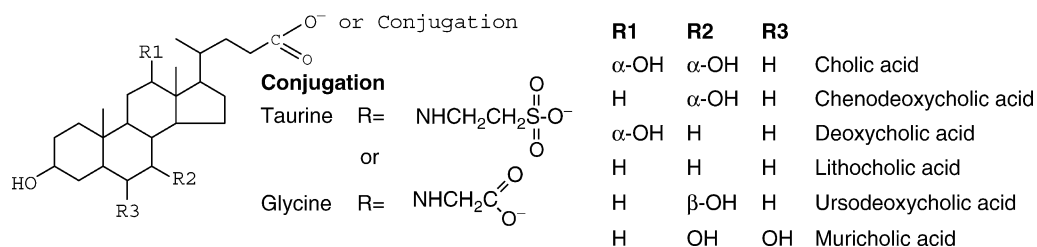
Basic Mechanisms

Gall or bile consists of bile acids, ►**phosphatidylcholine**, cholesterol and waste products and is secreted from the ►**hepatocytes** into the bile canaliculi ultimately ending in the hepatic duct. Most of the bile acids are present within the ►**enterohepatic** organs. About one-half of the secreted bile is diverted to the cystic duct and stored in the gallbladder. When a meal is ingested, bile from the gallbladder flows into the duodenum and intestine. The bile acids are efficiently (95%) absorbed again by passive diffusion and active transport in the terminal ileum, and transported back to the liver via the portal vein. In the liver, the bile acids are taken up at the basolateral (sinusoidal) membrane and exported again at the apical (canalicular) membrane of the hepatocytes into the bile canaliculus (transhepatic bile acid flux). This completes their enterohepatic circulation. Each

bile acid molecule may complete multiple cycles between liver and intestine per day. Because of this efficient recirculation, only a small amount of the ►**bile acid pool** size is derived from de novo biosynthesis. The bile acid pool presents the total amount of bile acids within the enterohepatic circulation.

Bile acids contain a variable number of hydroxyl groups, which determines their hydrophobicity and physiological properties. Humans synthesize two bile acids, cholic acid (3 α ,7 α ,12 α -trihydroxy) and chenodeoxycholic acid (3 α ,7 α -dihydroxy), which are termed primary bile acids (Fig. 1). Before secretion into the bile these bile acids are conjugated to glycine or taurine. In mice, bile acids are almost exclusively conjugated to taurine, whereas in humans glycoconjugates are usually predominant. Conjugation is important because it lowers the pK_a of bile acids, giving the molecules a charge at physiological pH, which explains the synonym bile salts. Conjugation renders bile acids more soluble and prevents passive absorption. In the colon, bile acids are subjected to deconjugation and 7 α -dehydroxylation by several microbial enzymes resulting in secondary bile acids that can become part of the circulating bile acid pool. The secondary bile acids of cholic acid and chenodeoxycholic acid are deoxycholic acid (3 α ,12 α) and lithocholic acid (3 α) respectively (Fig. 1). Lithocholic acid is toxic and due to its insolubility only poorly absorbed. Other bile acids are ursodeoxycholic acid (3 α ,7 β), hyocholic acid (3 α ,6 α ,7 α) and muricholic acid. Ursodeoxycholic acid is a bile acid found in large quantities in bear bile, but it is also a secondary bile acid that occurs naturally in humans in low quantities. Hyocholic acid is considered to be specific for pig bile. Muricholic acid is the name for a group of trihydroxy bile acids found in rodents of which β -muricholic acid (3 α ,6 β ,7 β) is the most prominent. The other stereoisomers of muricholic acid are α -(3 α ,6 β ,7 α) and ω -muricholic acid (3 α ,6 α ,7 β).

Bile acid synthesis from cholesterol is the prime pathway for cholesterol catabolism. Cholesterol is converted into bile acids via multiple pathways which involve 17 different enzymes. Many of these enzymes are predominantly expressed in the liver and are localized in several different subcellular



Bile Acids. Figure 1 Structure of selected bile acids.

compartments. Approximately 500 mg of cholesterol is converted into bile acids each day in the adult human liver. Bile acid biosynthesis involves modification of the ring structure of cholesterol, oxidation, and shortening of the side chain, and finally conjugation of the bile acid with an amino acid. All these steps and the enzymes involved are reviewed in detail by Russell [1]. The intermediates and enzymes of the classic (or neutral pathway) are displayed in Fig. 2. The classic pathway is responsible for the majority of total bile acid synthesis. The rate of synthesis is determined by cholesterol 7 α -hydroxylase, which is the first enzyme in the pathway (Fig. 2). Expression levels of this enzyme, which is encoded by the gene *CYP7A1*, are highly regulated and show a strong diurnal rhythm.

Bile acids have multiple functions [2]. The first function of bile acids is in nutrition, because they are essential to solubilize dietary lipids and fat-soluble vitamins, which subsequently promotes absorption of these molecules in the digestive tract. The second function of bile acids is bile formation. Bile acids generate bile flow from the hepatocyte and are essential for the extraction of phosphatidylcholine. As such bile acids are crucial for the excretion of certain waste products, such as ►**bilirubin**, heavy metals, and drug metabolites, but also cholesterol. Therefore bile formation and bile acid biosynthesis constitute the major pathways for cholesterol excretion. Besides these classic roles in dietary lipid absorption, bile formation and cholesterol homeostasis, it is now evident that bile acids are also signaling molecules [3]. Two major signaling mechanisms have been identified. Bile acids are ligands for the ►**G-protein-coupled receptor** (GPCR) TGR5 and ►**nuclear receptors** such as farnesoid X receptor (FXR). GPCRs also known as seven transmembrane receptors, are a protein family of cell surface receptors that bind an extracellular ligand and transduce this signal into G-protein activation (intracellular signal). TGR5 plays a role in the determination of the composition of gallbladder bile. Nuclear receptors are a family of transcription factors, which contains 48 members in human and are involved in the control of numerous processes, including development and metabolism [4]. Nuclear receptors typically consist of a DNA-binding domain and a ligand-binding domain. The DNA binding domain binds specific so-called response elements in the promoter of target genes. Upon binding of an intracellular ligand, nuclear receptors change conformation which induces dissociation of corepressors and the recruitment of transcriptional cofactors, resulting in the activation of transcription. The discovery of bile acids as the endogenous FXR ligands suggested a function for them in the regulation of the enterohepatic circulation and biosynthesis of bile acids, which is in line with the reported expression pattern of FXR in liver and intestine. In these tissues,

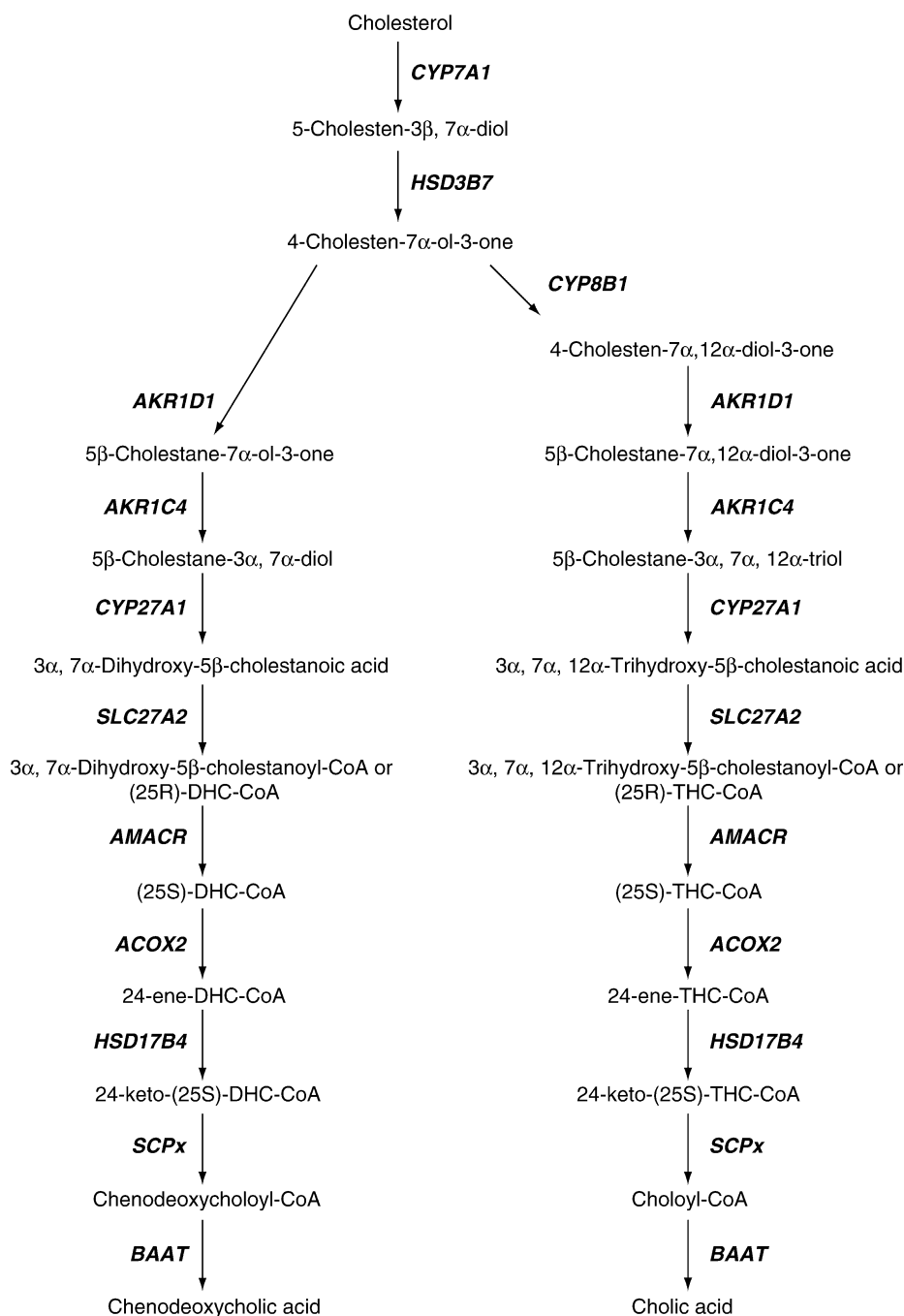
FXR activation protects against accumulation of bile acids, which is toxic (reviewed in [5]). To summarize (Fig. 3), FXR activation in the liver decreases bile acid biosynthesis and increases conjugation of bile acids followed by the excretion of bile acids from the hepatocyte into the bile canaliculus leading to an increase in the formation of bile. In the intestine, FXR activation decreases hepatic bile acid biosynthesis and increases expression of a protective bile acid binding protein and the basolateral bile acid transporters. Of particular interest is the FXR-mediated induction of the expression of short heterodimer partner (SHP) in the liver and fibroblast growth factor 19 (FGF19) in the ileum. SHP is an atypical nuclear receptor that only has a ligand-binding domain and no DNA-binding domain and inhibits the activity of several nuclear receptors. SHP induction underlies the negative feedback regulation of bile acid biosynthesis because it potently inhibits the expression of *CYP7A1*, the rate-limiting enzyme in bile acid biosynthesis. FGF19 is a secreted peptide hormone that signals to the liver and the gallbladder. In the liver, FGF19 signaling decreases expression of *CYP7A1*. FGF19 also decreases smooth muscle tension in the gallbladder allowing filling with and storage of bile. During storage, the bile is concentrated by water removal in order for bile acids to reach concentrations in the intestinal lumen high enough to solubilize dietary lipids.

In addition to regulation of their own enterohepatic circulation, bile acids can also regulate cholesterol, triglyceride, energy, and glucose homeostasis (reviewed in [3]). Bile acids decrease their own biosynthesis and as such increase hepatic and LDL-cholesterol. Interestingly bile acids also affect triglyceride homeostasis. In fact, for a long time it has been known that in man there is an inverse relationship between bile acids and hepatic VLDL production. Treatment with bile acid-binding resins, ileal exclusion or bile withdrawal interrupt the enterohepatic circulation and induce the production of VLDL. Conversely, treatment of cholesterol gallstones with chenodeoxycholic acid increases the bile acid pool and reduces hypertriglyceridemia. All these effects have been attributed to FXR-mediated downregulation of bile acid biosynthesis and lipogenesis. In mice, bile acids have been reported to inhibit diet-induced obesity and prevent the development of insulin resistance by increasing energy expenditure in brown adipose tissue via the activation of TGR5.

Pharmacological Intervention

Several pharmacological interventions target bile acid homeostasis.

Gallstones. Bile acids keep cholesterol soluble in gallbladder bile. Therefore, they are used for the dissolution of cholesterol gallstones. Initial treatment

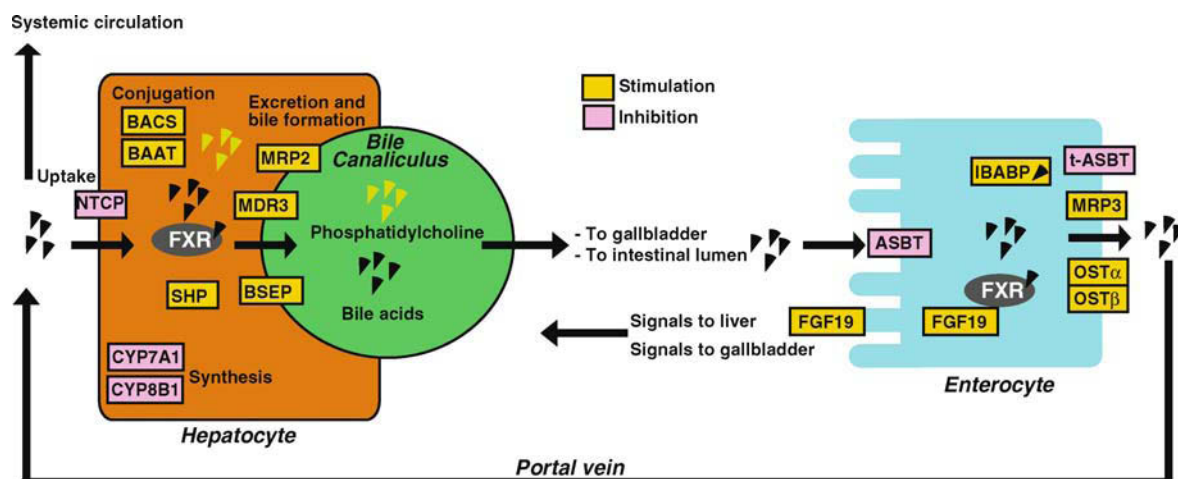


Bile Acids. Figure 2 Schematic representation of the enzymes and intermediates in the classic bile acid biosynthesis pathway, which is responsible for ~90% of the total bile acid synthesis humans. *CYP7A1* encodes cholesterol 7 α -hydroxylase; *HSD3B7* encodes 3 β -hydroxy- Δ^5 - C_{27} -steroid oxidoreductase; *CYP8B1* encodes sterol 12 α -hydroxylase; *AKR1D1* encodes Δ^4 -3-oxosteroid 5 β -reductase; *AKR1C4* encodes 3 α -hydroxysteroid dehydrogenase; *CYP27A1* encodes sterol 27-hydroxylase; *SLC27A2* encodes bile acyl-CoA synthetase; *AMACR* encodes α -methylacyl-CoA racemase; *ACOX2* encodes branched-chain acyl-CoA oxidase; *HSD17B4* encodes D-bifunctional protein; *SCPx* encodes sterol carrier protein X; *BAAT* encodes bile acyl-CoA:amino acid *N*-acyltransferase.

with chenodeoxycholic acid was hepatotoxic and was replaced by treatment with ursodeoxycholic acid.

Hyperlipidemia. Bile acid binding resins such as cholestyramine sequester bile acids in the intestine,

preventing their uptake in the ileum and consequently their enterohepatic recirculation. Fecal loss of bile acids is compensated by increased biosynthesis of bile acids from cholesterol. Cholestyramine treatment therefore reduces



Bile Acids. Figure 3 FXR target genes involved in the enterohepatic recycling and detoxification of BAs. Genes whose expression is directly induced by bile acids and FXR are in yellow rectangles; those in pink rectangles are inhibited by bile acids. Abbreviations not used in Fig. 2 are: BACS (SLC27A2), bile acyl-CoA synthetase; BSEP, bile salt export pump; MDR3, multidrug resistance 3 p-glycoprotein; MRP2, multidrug resistance-associated protein 2; IBABP, ileal bile acid binding protein; NTCP, sodium taurocholate cotransporting polypeptide; ASBT, apical sodium dependent bile salt transporter; FGF, fibroblast growth factor; OST, organic solute transporter.

plasma cholesterol and was used to treat hypercholesterolemia before *statins* became available.

As described in the previous section, bile acids have evolved over the last years from regulators of bile acid homeostasis to general metabolic integrators. It is therefore not too surprising that a number of bile acid-activated signaling pathways have become attractive targets for the treatment of gallstones and other metabolic diseases, such as obesity, type 2 diabetes, hyperlipidemia, and atherosclerosis.

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Bimodal Distribution

A bimodal distribution is a frequency distribution of a certain phenotype with two peaks separated by an antimode.

► Pharmacogenetics

Bioavailability

Bioavailability is the amount of drug in a formulation that is released and becomes available for absorption or the amount of the drug absorbed after oral administration compared to the amount absorbed after intravenous administration (bioavailability = 100%), judged from areas remaining under plasma drug concentration-time curves.

- Area under the Curve (AUC)
- Pharmacokinetics
- Catechol-O-Methyltransferase and its Inhibitors

Bile Salts

► Bile Acids

Biochip

► Microarray Technology

Biocomputing

- Bioinformatics
- Molecular Modelling

Biogenic Amines

Definition

Acetylcholine, serotonin, norepinephrine, epinephrine, dopamine, histamine and polyamines (spermine/putrescin) are often collectively referred to as biogenic amines. These agents play key roles in neurotransmission and other signaling functions. They are relatively small in size and contain a protonated amino group or a permanently charged ammonium moiety. Biogenic amines are synthesized in nerve cells from amino acids. They are released from vesicles localized in presynaptic terminals into the synaptic cleft. Biogenic amines bind to cell membrane located receptors at postsynaptic terminals. The synaptic cleft is cleared of biogenic amines through reuptake transporters located at the presynaptic terminal or by enzymes degrading the amines.

- Muscarinic Receptors
- Serotoninerigic System
- α -Adrenergic System
- β -Adrenergic System
- Dopamine System
- Histaminergic System
- Antidepressant Drugs
- Orexins
- Trace Amines

Bioinformatics

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Synonyms

Computational biology; Computational molecular biology; Biocomputing; in silico biology

Definition

Bioinformatics generates knowledge from computer analysis of biological data. These can consist of the information stored in the genetic code, but also experimental results from various sources, patient statistics, and scientific literature. Research in bioinformatics includes method development for storage, retrieval, and analysis of the data. Bioinformatics is a rapidly developing branch of biology and is highly interdisciplinary, using techniques and concepts from informatics, statistics, mathematics, chemistry, biochemistry, physics, and linguistics. It has many practical applications in different areas of biology and medicine.

Description

The history of computing in biology goes back to the 1920s when scientists were already thinking of establishing biological laws solely from data analysis by induction (e.g. A.J. Lotka, *Elements of Physical Biology*, 1925). However, only the development of powerful computers, and the availability of experimental data that can be readily treated by computation (e.g. DNA or amino acid sequences and 3D structures of proteins) launched bioinformatics as an independent field. Today, practical applications of bioinformatics are readily available through the World Wide Web, and are widely used in biological and medical research. As the field is rapidly evolving, the very definition of bioinformatics is still the matter of some debate.

The relationship between computer science and biology is a natural one for several reasons. First, the phenomenal rate of biological data being produced provides challenges: massive amounts of data have to be stored, analysed, and made accessible. Second, the nature of the data is often such that a statistical method, and hence computation, is necessary. This applies in particular to the information on the building plans of proteins and of the temporal and spatial organisation of their expression in the cell encoded by the DNA. Third, there is a strong analogy between the DNA sequence and a computer program (it can be shown that the DNA represents a ►turing machine).

Analyses in bioinformatics focus on three types of datasets: genome sequences, macromolecular structures, and functional genomics experiments (e.g. expression data, yeast two-hybrid screens). But bioinformatic analysis is also applied to various other data, e.g. taxonomy trees, relationship data from metabolic pathways, the text of scientific papers, and patient statistics. A large range of techniques are used, including primary sequence alignment, protein 3D structure alignment, phylogenetic tree construction, prediction and classification of protein structure, prediction of RNA structure, prediction of protein function, and expression data clustering. The emergence of systems biology as a new branch in biology has further increased

the role of computing, and the term “computational biology” often refers to the calculation of systems behaviour. Algorithmic development is an important part of bioinformatics, and techniques and algorithms were specifically developed for the analysis of biological data (e.g. the ►dynamic programming algorithm for sequence alignment).

Bioinformatics has a large impact on biological research. Giant research projects such as the human genome project [1] would be meaningless without the bioinformatics component. The goal of sequencing projects, for example is not to corroborate or refute a hypothesis, but to provide raw data for later analysis. Once the raw data are available, hypotheses may be formulated and tested *in silico*. In this manner, computer experiments may answer biological questions which cannot be tackled by traditional approaches. This has led to the founding of dedicated bioinformatics research groups as well as to a different work practice in the average bioscience laboratory where the computer has become an essential research tool.

Three key areas are the organisation of knowledge in databases, sequence analysis, and structural bioinformatics.

Organising Biological Knowledge in Databases

Biological raw data are stored in public ►databanks (such as Genbank or EMBL for primary DNA sequences). The data can be submitted and accessed via the World Wide Web. Protein sequence databanks like trEMBL provide the most likely translation of all coding sequences in the EMBL databank. Sequence data are prominent, but also other data are stored, e.g. yeast two-hybrid screens, expression arrays, systematic gene-knock-out experiments, and metabolic pathways.

The stored data need to be accessed in a meaningful way, and often contents of several databanks or databases have to be accessed simultaneously and correlated with each other. Special languages have been developed to facilitate this task (such as the Sequence Retrieval System (SRS) and the Entrez system). An unsolved problem is the optimal design of inter-operating database systems. ►Databases provide additional functionality such as access to sequence homology searches and links to other databases and analysis results. For example, SWISSPROT [2] contains verified protein sequences and more annotations describing the function of a protein. Protein 3D structures are stored in specific databases (e.g. the Protein Data Bank [3], now primarily curated and developed by the Research Collaboratory for Structural Bioinformatics). Organism specific databases have been developed (such as ACEDB, the A C. Elegans Database for the *C. elegans* genome, FLYBASE for *D. melanogaster* etc.). A major problem is errors in

databanks and databases (mostly errors in annotation) in particular since errors propagate easily through links.

Also databases of scientific literature (such as PUBMED, MEDLINE) provide additional functionality, e.g. they can search for similar articles based on word-usage analysis. Text recognition systems are being developed that automatically extract knowledge about protein function from the abstracts of scientific articles, notably on protein–protein interactions.

Analysing Sequence Data

The primary data of sequencing projects are DNA sequences. These become only really valuable through their annotation. Several layers of analysis with bioinformatics tools are necessary to arrive from a raw DNA sequence at an annotated protein sequences:

- Establish the correct order of ►sequence contigs to obtain one continuous sequence
- Find the translation and transcription initiation sites, find promoter sites, define ►open reading frames (ORF)
- Find splice sites, introns, exons
- Translate the DNA sequence into a protein sequence, searching all six frames
- Compare the DNA sequence to known protein sequences to verify exons, etc. with homologous sequences

Some completely automated annotation systems have been developed (e.g. GENEQUIZ), which use a multitude of different programs and methods.

The protein sequences are further analysed to predict function. The function can often be inferred if a sequence of a ►homologous protein with known function can be found. Homology searches are the predominant bioinformatics application, and very efficient search methods have been developed [4]. The often difficult distinction between ►orthologous sequences and ►paralogous sequences facilitates the functional annotation in the comparison of whole genomes. Several methods detect glycosylation, myristylation and other sites, and the prediction of signal peptides in the amino acid sequence give valuable information about the subcellular location of a protein.

The ultimate goal of sequence annotation is to arrive at a complete functional description of all genes of an organism. However, function is an ill-defined concept. Thus, the simplified idea of “one gene – one protein – one structure – one function” cannot take into account proteins that have multiple functions depending on context (e.g. subcellular location and the presence of cofactors). Well-known cases of “moonlighting” proteins are lens crystalline and phosphoglucose isomerase. Currently, work on ►ontologies is under way to explicitly define a vocabulary that can be applied to all

organisms even as knowledge of gene and protein roles in cells is accumulating and changing.

Families of similar sequences contain information on sequence evolution in the form of specific conservation patterns at all sequence positions. Multiple sequence alignments are useful for

- Building ►sequence profiles or ►Hidden Markov Models to perform more sensitive homology searches. A sequence profile contains information about the variability of every sequence position, improving structure prediction methods (secondary structure prediction). Sequence profile searches have become readily available through the introduction of PsiBLAST [4]
- Studying evolutionary aspects, by the construction of phylogenetic trees from the pairwise differences between sequences: for example, the classification with 70S, 30S RNAs established the separate kingdom of archaea
- Determining active site residues, and residues specific for subfamilies
- Predicting protein–protein interactions
- Analysing ►single nucleotide polymorphisms to hunt for genetic sources of diseases

Many complete genomes of microorganisms and a few of eukaryotes are available [1]. By analysis of entire genome sequences a wealth of additional information can be obtained. The complete genomic sequence contains not only all protein sequences but also sequences regulating gene expression. A comparison of the genomes of genetically close organisms reveals genes responsible for specific properties of the organisms (e.g. infectivity). Protein interactions can be predicted from conservation of gene order or operon organisation in different genomes. Also the detection of gene fusion and gene fission (i.e. one protein is split into two in another genome) events helps to deduce protein interactions.

Structural Bioinformatics

This branch of bioinformatics is concerned with computational approaches to predict and analyse the spatial structure of proteins and nucleic acids. Whereas in many cases the primary sequence uniquely specifies the 3D structure, the specific rules are not well understood, and the ►protein folding problem remains largely unsolved. Some aspects of protein structure can already be predicted from amino acid content. Secondary structure can be deduced from the primary sequence with statistics or ►neural networks. When using a multiple sequence alignment, secondary structure can be predicted with an accuracy above 70%.

Three-dimensional models can be obtained most easily if the 3D structure of a homologous protein is known (homology modelling, comparative modelling). A homology model can only be as good as the sequence

alignment: whereas protein relationships can be detected at the 20% identity level and below, a correct sequence alignment becomes very difficult, and the homology model will be doubtful. From 40 to 50% identity the models are usually mostly correct; however, it is possible to have 50% identity between two carefully designed protein sequences with different topology (the so-called JANUS protein). Remote relationships that are undetectable by sequence comparisons may be detected by sequence-to-structure-fitness (or ►threading) approaches: the search sequence is systematically compared to all known protein structures. Ab initio prediction of protein 3D structure remains the major challenge; some progress has been made recently by combining statistical with ►force-field based approaches.

Membrane proteins are interesting drug targets. It is estimated that membrane receptors form 50% of all drug targets in pharmacological research. However, membrane proteins are underrepresented in the PDB structure database. Since membrane proteins are usually excluded from structural genomics initiatives due to technical problems, the prediction of transmembrane helices and solvent accessibility is very important. Modern methods can predict transmembrane helices with a reliability greater than 70%.

Understanding the 3D structure of a macromolecule is crucial for understanding its function. Many properties of the 3D structure cannot be deduced directly from the primary sequence. Obtaining better understanding of protein function is the driving force behind structural genomics efforts, which can be thus understood as part of functional genomics. Similar structure can imply similar function. General structure-to-function relationships can be obtained by statistical approaches, for example by relating secondary structure to known protein function or surface properties to cell location.

The increased speed of structure determination necessary for the structural genomics projects makes an independent validation of the structures (by comparison to expected properties) particularly important. Structure validation helps to correct obvious errors (e.g. in the covalent structure) and leads to a more standardised representation of structural data, e.g. by agreeing on a common atom name nomenclature. The knowledge of the structure quality is a prerequisite for further use of the structure, e.g. in molecular modelling or drug design.

In order to make as much data on the structure and its determination available in the databases, approaches for automated data harvesting are being developed. Structure classification schemes, as implemented for example in the SCOP, CATH, and FSSP databases, elucidate the relationship between protein folds and function and shed light on the evolution of protein domains.

Combined analysis of structural and genomic data will certainly get more importance in the near future. Protein folds can be analysed for whole genomes.

Protein–protein interactions predicted on the sequence level can be studied in more detail on the structure level. ► **Single Nucleotide Polymorphisms** can be mapped on 3D structures of proteins in order to elucidate specific structural causes of disease.

More detailed aspects of protein function can be obtained also by ► **force-field** based approaches. Whereas protein function requires protein dynamics, no experimental technique can observe it directly on an atomic scale, and motions have to be simulated by molecular dynamics (MD) simulations. Also free energy differences (e.g. between binding energies of different protein ligands) can be characterised by MD simulations. Molecular mechanics or molecular dynamics based approaches are also necessary for homology modelling and for structure refinement in X-ray crystallography and NMR structure determination.

Drug design exploits the knowledge of the 3D structure of the binding site (or the structure of the complex with a ligand) to construct potential drugs, for example inhibitors of viral proteins or RNA. In addition to the 3D structure, a ► **force-field** is necessary to evaluate the interaction between the protein and a ligand (to predict binding energies). In virtual screening, a library of molecules is tested on the computer for their capacities to bind to the macromolecule.

Pharmacological Relevance

Many aspects of bioinformatics are relevant for pharmacology. Drug targets in infectious organisms can be revealed by whole genome comparisons of infectious and non-infectious organisms. The analysis of single nucleotide polymorphisms reveals genes potentially responsible for genetic diseases. Prediction and analysis of protein 3D structure is used to develop drugs and understand drug resistance.

Patient databases with genetic profiles, e.g. for cardiovascular diseases, diabetes, cancer, etc. may play an important role in the future for individual health care, by integrating personal genetic profile into diagnosis, despite obvious ethical problems. The goal is to analyse a patient's individual genetic profile and compare it with a collection of reference profiles and other related information. This may improve individual diagnosis, prophylaxis, and therapy.

► Molecular Modelling

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Biological Medicines

► Biologicals

Biological Therapeutics

► Biologicals

Biologicals

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Synonyms

Biological medicines; Biological therapeutics; Blood products; Vaccines; Diagnostics

Definition

Biologicals are pharmaceutical agents derived or synthesized from living organisms and/or their products used for diagnostic, preventive, and therapeutic purposes necessary in the practice of human and veterinary medicine. Blood and blood products form a special category of biologicals as they are derived from repeated donation of large amounts of blood or plasma by healthy volunteers. Unlike the characterization of traditional pharmaceuticals, however, biologicals are

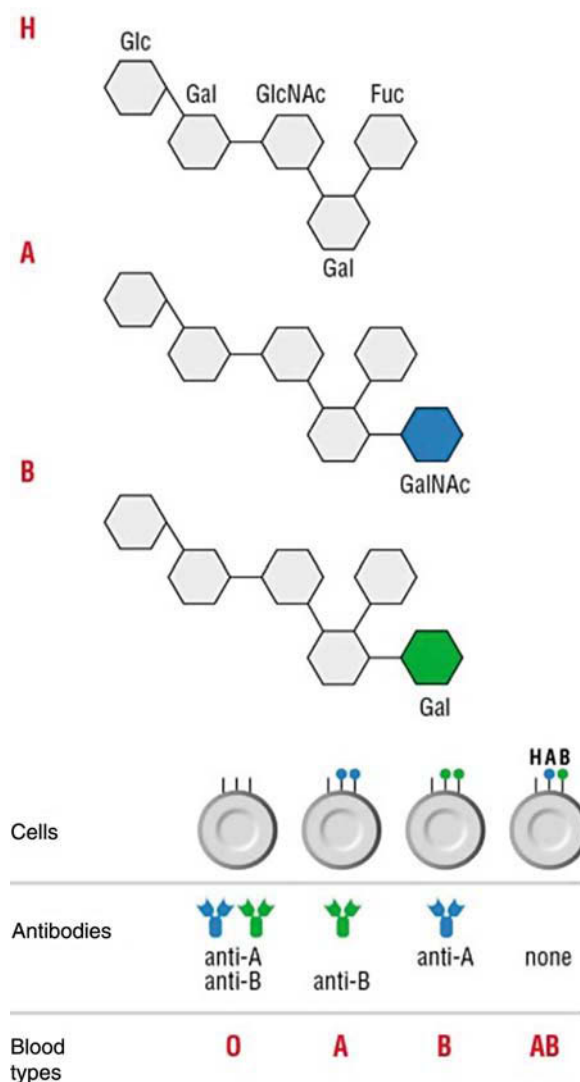
more difficult to characterize because of the complex molecular structure of their components and their interactions with agents used during their manufacture and/or that are present in the final products, e.g., additives, stabilizers, preservatives, and adjuvants. Thus, product safety, efficacy, and quality assurance are carried out by rigorous biological standardization that must rely on consistency approach in the manufacturing of products and their comparability with reference materials used worldwide as standards by the regulatory authorities.

Mechanisms of Action

Blood and Blood Products

The discovery of ABO blood groups at the beginning of the 20th century initiated transfusion of donated human blood to replace acute blood loss suffered by patients with trauma or undergoing major surgical procedures [1]. The molecular basis of genetically determined ABH antigens and the corresponding appearance of naturally occurring antibodies in the serum for an antigen absent on the cell are illustrated in Fig. 1, which is adapted from [2]. The terminal sugars, enzymatically synthesized by genetically determined transferases, determine the antigenic specificity of the ABH antigens on cells. Reciprocally, the serum naturally contains isoantibodies against the antigens absent on the red cells; thus, group O individuals have absence of both A and B antigens on the red cell membrane and hence have anti-A and anti-B antibodies present in the serum. With subsequent discoveries of Rh and other blood groups, blood typing and pretransfusion compatibility testing or cross matching became so perfected that most serious hemolytic transfusion reactions are exceedingly rare among an estimated 25 million patients transfused worldwide each year. Landsteiner received the Nobel Prize in 1943 for his discovery of blood groups, making transfusion the most widely used procedure in clinical practice.

Blood centers collect whole blood from voluntary donors and generally divide each donation into components, viz., red cells, platelets, and leukocytes, and fresh frozen plasma (FFP) for transfusion therapy for specific diseases (see later in the section on Clinical Use). Therapeutic plasma proteins, e.g., albumin, gamma globulins, and factor VIII are derived from the plasma procured at plasmapheresis centers. Batches of several thousand liters of plasma are processed by Cohn Fractionation, which uses discrete concentrations of alcohol to precipitate and separate the principal plasma proteins (albumin and gamma globulins). Human gamma globulins (Ig) get aggregated in the Cohn fractionation and require disaggregating accomplished at low pH or by enzymatic digestion to render Ig safer for intravenous use as IVIg. Modified Cohn fractionation and chromatographic separation methods have been developed



Biologicals. Figure 1 The ABO blood group system with immunochemical specificity due to terminal sugars and reciprocal antibodies in the serum. From *Immunity: Immune Response in Inflammatory Disease* by DeFranco, Locksley and Robertson [2].

to derive not only albumin, globulins, but also clotting factor concentrates (factor VII, VIII, and IX), fibrinogen and alpha-1 antitrypsin. Recombinant DNA technology for expressing the genes encoding each of the major therapeutic plasma proteins, viz., albumin, alpha-1-antitrypsin, and clotting factors VII, VIII, and IX is feasible for providing molecularly homogeneous, clinically useful and safe blood products for transfusion therapy. However, molecularly derived products are disproportionately costly and not competitive with plasma-derived therapeutic proteins. Humanized monoclonal antibodies produced by recombinant DNA technology, e.g., hepatitis B immune globulins (HBIG) or

Rh immune globulin for intravenous use may have a place in specific transfusion therapies.

Cellular ►**cytokines** (interferons, G-CSF) and immune response modifiers originally produced from human cells, most often leukocytes, have now been replaced with recombinant products with well-defined structure/function. Futuristic advances in experimental hematology portend development of human blood cells produced from the hemopoetic stem cells. Yet for the foreseeable future, homologous blood donated by healthy, altruistic voluntary blood donors remains the principal source of safe and adequate supply of blood and blood products for transfusion therapy.

Diagnosics

In vitro or in vivo laboratory tests are tools or procedures used as part of the diagnosis process in which a physician seeks to determine the physiological or biochemical cause(s) of a patient's disease symptoms. While clinical diagnosis performed in the physician's office is based on clinical history, physical examination and measurement of blood pressure, heart rate, etc., additional lab tests are required to evaluate a list of clinical probabilities. Depending on the type of test, biological products may be used to evaluate the patient's tissues, such as blood, urine, saliva, DNA, hair, or stool, by utilizing specific interactions between the biological diagnostic and target or substance of interest. A majority of currently available tests for in vitro diagnosis (IVD) are based on hematological, microbiological, biochemical, and immunological analysis of patient specimens. This medical discipline is commonly referred to as laboratory medicine or clinical pathology.

Many of the laboratory tests are based on immunoassays for antigens/antibodies present in the patient's test specimens, by having a biologically standardized set of antigens or antibodies for the detection and quantification of corresponding analytes for IVD. Continuing expansion in the fundamental knowledge about cell biology, cell differentiation antigens (nearly 400), cellular receptors and cytokines, and various growth factors in health and disease has led to availability of both polyclonal and monoclonal antibodies used for IVD.

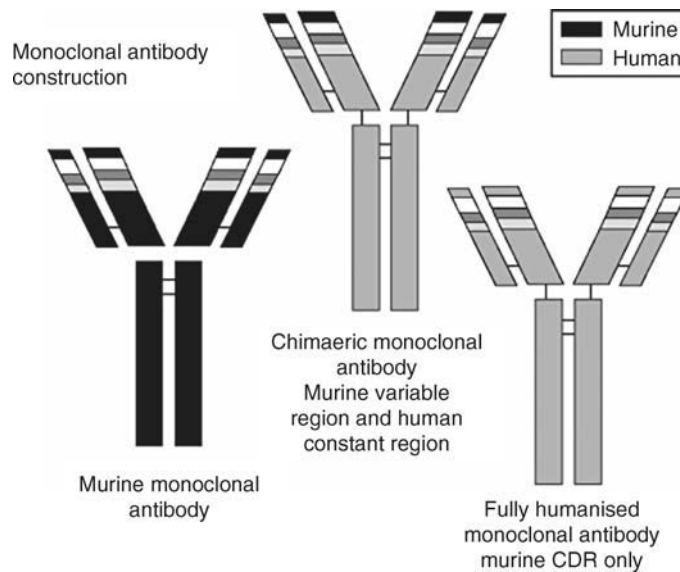
Therapeutics

Simply speaking, a therapeutic biological used for healing or treating any ailment is a product derived from another living being, and therefore falls under the rubric of biologicals; they are often referred to as biological medicines or biotherapeutic drugs. Recombinant DNA technology enabled expression of cloned genes to produce therapeutic proteins or biological medicines.

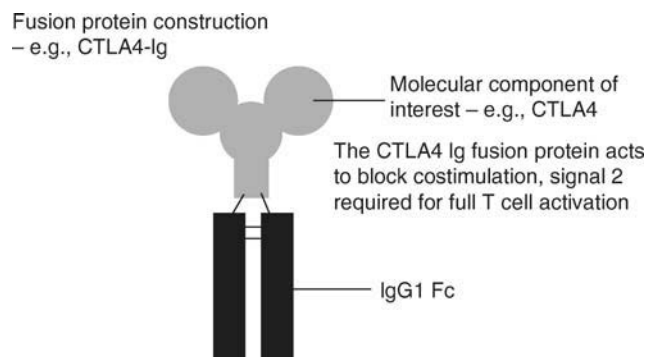
Major advances made in our understanding of the immune system and disease pathogenesis have

coincided with the development of several biological medicines developed during the past two decades. Cytokines, monoclonal antibodies, and cellular therapies offer promising treatments for a variety of human cancers, persistent infections, and tissue and organ transplantation. The dynamic field of immune therapies and cellular therapies is still evolving, rapidly developing, and most promising in the field of cancer and immune disorders. Principal promises pertain to the following:

- Besides the plasma-derived immunoglobulins for antibody therapies listed in the foregoing section on blood components, a variety of monoclonal antibodies and fusion proteins are prepared by molecular methods using recombinant DNA technology. The mouse monoclonal antibodies first used for therapeutic purposes could not be repeatedly given because they induce human antibodies against mouse IgG (Fc). This is overcome by genetic constructs of chimera for humanized monoclonal antibodies and for novel fusion proteins containing the constant region of IgG heavy chains (Fc) of human origin as illustrated in Figs. 2 and 3, reproduced from Johnston [3].
- The biological agents that directly or indirectly mediate beneficial effects against patient's persistent infection or response to a neoplasm are a class of biologicals termed cytokines or biological response modifiers. They include interferons (alpha, beta, and gamma), interleukins (total of 33 and growing), tumor necrosis factors, hemopoetic growth factors (granulocyte/monocyte colony stimulating factors, G/GM/M-CSF), stem cell factor (SCF), and a variety of specific monoclonal antibodies. While some of the crude cytokines such as interferons were originally derived from human leukocytes and their subsets, the recombinant DNA technology has tremendously facilitated the identification and production of a large number of the human proteins with potent effects on the function and growth of normal and neoplastic cells.
- Cellular therapies in transplantation and cancer are based on specific cells separated or sorted from human blood, bone marrow, or cord blood by means of their specific cell surface markers or cell differentiation antigens, e.g., CD3, CD4, CD8, CD14, CD19, and CD34. For example, the CD34⁺ stem cells, especially those derived from human embryos, have the capacity to differentiate in culture to generate different somatic cells, e.g., liver cells, heart cells, neurons, etc. This exploding field of research is now termed regenerative medicine.
- ►**Gene therapy** uses cloned DNA in adeno or retroviral vectors to transduce host cells for long-term therapy of certain genetic disorders with defined



Biologicals. Figure 2 Monoclonal antibody construction: the different components of the antibody structure can be derived from different genetic sources of B cells. Chimeric monoclonal antibodies are derived from mouse variable-region genetic sequences combined with constant-region genes of human origin. Fully humanized monoclonal antibodies are composed of mouse complementarity-determining regions (CDRs), the rest of the antibody being of human genetic origin. Fully human monoclonal antibodies are produced from entirely human genetic sequences.



Biologicals. Figure 3 Fusion protein construction: combination of the molecular component of interest with the constant region (Fc) of an antibody molecule, usually immunoglobulin (Ig)G1 Fc, imparts the Fc function on to the molecular component for therapeutic use. The example given is of CTLA4-Ig, derivatives of which have shown clinical efficacy in the treatment of rheumatoid arthritis and transplant rejection.

molecular mechanisms, e.g., severe combined immunodeficiency resulting from T cell defect due to adenosine deaminase deficiency. However, evolution of leukemia and lymphoma in some of the treated patients has dampened this novel therapy.

Vaccines

Vaccines are biologicals used as agents for disease prevention. Unlike their synthetic counterparts, biologically derived vaccines are made from live disease-causing agents or their products, conferring immunity against the disease each organism causes.

A successful vaccine stimulates an effective adaptive immune response directed at appropriate target antigens without causing disease. Edward Jenner achieved this through the use of the animal virus vaccinia to immunize against the related human smallpox virus. Because the two viruses are closely related, they can be recognized by the same immune cells but unlike the smallpox virus vaccinia does not cause disease in humans. Thus the vaccine is both antigenic and safe. Another crucial property of vaccinia virus is that as a natural pathogen it contains components that are recognized by the cells of the innate immune system and is thus able to induce the production of cytokines and activation of dendritic cells,

which are required to launch an effective adaptive immune response: that is, it is immunogenic.

Introduction of an antigen into the bloodstream leads to activation of the lymphocytes involved in active immunity. Special cells called antigen-presenting cells (APC) process the pathogen and present pieces of it to T cells, which are primarily involved in cell-mediated immunity, in order to initiate T cell activation. B cells, on the other hand, can interact directly with antigens in the bloodstream via surface molecules called antibodies that are specifically tailored to recognize particular nonself molecules. Once this occurs, a T cell activates the B cell, causing it to produce millions of copies of the antibody that binds the antigen; this is known as the humoral immune response. Certain offspring of dividing B and T cells will become a special cellular class known as memory cells, which can then induce a faster and fiercer immune response the next time that particular pathogen is encountered.

Clinical Uses (Including Side Effects)

Blood and Blood Products

Transfusion Therapy

Blood and blood products are used in transfusion medicine to replenish the depletion of any formed element of blood in order to optimally maintain physiological functions. There is no substitute for human blood used in life-saving transfusions employed in clinical practice. The World Health Organization (WHO) estimates that 81 million pints (500 ml units) of blood were collected worldwide in 2005. Because an average receives 3.8 units of blood, one can estimate that at least 21 million patients in the world are transfused each year; however, less blood is available for transfusion in developing countries, hence annually 25 million patients are estimated worldwide to receive blood transfusions. Of the 81 million donations, only 38% were collected in developing countries where 82% of the world population resides. It is also estimated that developing countries have access to only 20% of the world's safe blood, tested adequately for transfusion-transmitted infections (TTI), viz., hepatitis B and C (HBV, HCV) and HIV. Blood donated by healthy volunteers in North America, Europe, and Japan is screened by molecular amplification using polymerase chain reaction (PCR) or transcription-mediated amplification (TMA) of the TTI agents' genomes. Safer blood supply is a compelling need worldwide but yet unmet and hence a topic of considerable debate and current research. The frequency of TTI in developed nations is less than one in a million units of blood.

Blood Component Therapy

Each unit of blood (450 ml blood + 75 ml preservative anticoagulant solution) donated for transfusion can be divided into packed red cells used for adequate tissue

oxygenation, platelet concentrates for hemostasis, and FFP for maintaining oncotic pressure and providing coagulation factors. Whole blood can be donated five times a year by healthy volunteers. Plateletpheresis (selective removal of platelets) can be performed on blood donors at least twice a week to provide large dose of single donor platelets for patients with thrombocytopenia requiring repeated infusion. Plasmapheresis (selective removal of plasma) can be performed on healthy adults once or twice a week. This procedure permits plasma collection in large amounts for fractionation of albumin, globulins, and cryoprecipitated or purified factor VIII for maintenance therapy in hemophilia. The treatment of hemophilia with purified factor VIII principally drives procurement of plasma by plasmapheresis, supplemented with unused FFP. While human albumin (5% solution) is used as a blood volume expander, gamma globulin (5–16% solution) is most commonly used for passive prophylaxis against hepatitis A and treatment of various hematological and immunological disorders [1]. Pathogen-specific hyperimmune globulins are derived by plasmapheresis of immunized donors or convalescent patients, e.g., HBIG or Zoster immune globulins.

Adverse Effect of Transfusion

Rare hemolytic transfusion reactions are often due to human errors, which result in transfusion of blood to wrong patients. Many hemolytic transfusion reactions culminate in renal shut down with acute tubular necrosis; this is totally reversible with hemodialysis as needed until the renal function is spontaneously restored in less than 2 weeks. Thus, acute tubular necrosis in hemolytic transfusion reaction is totally reversible. Nonhemolytic transfusion reactions in contrast are commonly encountered in 1–2% of transfusion recipients and characterized by fever, urticaria, graft versus host reaction, transfusion-related acute lung injury (TRALI), or rare anaphylactic reactions. These nonhemolytic reactions are due to incompatibility with the formed elements of blood other than red cells. The immune and nonimmune mechanisms underlying nonhemolytic transfusion reactions are often difficult to define and are empirically managed in clinical practice [1].

Diagnostics

Because the sheer magnitude of the tests performed in laboratory diagnosis is so great, e.g., over 3,600 tests in the alphabetical index of the laboratory tests offered by UCLA Medical Center [4], a single example will be illuminated to demonstrate a particular use of biologically derived diagnostics in home pregnancy test.

Pregnancy Test: An Illustrative Example

The classic “pee on a stick” variety of pregnancy test remains the most common among the tests for human

chorionic gonatotropin (hCG) hormone, which is usually found in the blood and urine within a week of embryonic implantation into the uterus. This test uses a series of three antibody sets: two from mice and one from goats. Prior to manufacturing the tests themselves, mice are injected with hCG, a foreign antigen, inducing their immune system to elicit antibodies against hCG that are then harvested and purified. Similarly, goats are immunized with mouse immune globulins to elicit antimouse IgG antibodies.

When someone takes a pregnancy test, they first urinate on the end of the stick. Here, mobile mouse anti-hCG antibodies will travel up the stick with the urine, some of which will bind to hCG if it is present. Once the urine reaches the test region, it will encounter a second set of mouse anti-hCG molecules, this time bound to the test strip (stationary). Mouse antibodies that have bound hCG will be caught by the stationary antibodies, creating a literal hCG “sandwich.” Mobile mouse antibodies that did not encounter hCG (there are always more antibodies than hCG molecules) travel with the urine to the control region, where they are captured by goat anti-mouse antibodies. In both the test and control regions, the mobile mouse antibodies induce a color change in an indicator that has been inlaid in a straight line – hence the characteristic two-line system.

In general, diagnostic tests that look for a particular protein of interest use biologically derived antibodies, usually from mice. However, proteins, DNA, RNA, and other biologicals may be derived from a variety of organisms like bacteria, yeast, plants, and other mammals for use as diagnostics.

Therapeutics

- **►Cytokines** and biological response modifiers represent a broad class of therapeutic agents that modify the hosts’ response to cancer or cancer therapies. The enormous body information about their clinical uses and their side effects is beyond the scope of this essay that can only give illustrative examples. For an up-to-date information the reader can resort to reference [5]. As many as 33 different interleukins are known and the list continues to grow; IL-2 used in the treatment of kidney cancer is one example. Interferon alpha is used for chronic myelogenous leukaemia, hairy cell leukaemia and Kaposi’s sarcoma. Interferons are also used in the treatment of chronic infections such as viral hepatitis. Tumor necrosis factor (alpha), G/GM/M-CSF, and several other cellular factors are used in treatment of various cancers. Many of these cytokines produce serious side effects that limit their use.
- The list of MoAb or fusion proteins used in treatment of every type of cancer is continually growing as research identifies more cancer-associated antigens. MoAb targeting a tumor-specific antigen is used

in two forms: (i) Naked, without any drug or radioactive material attached, e.g., Panitumumab for colorectal cancers or Trastuzumab for breast cancer; (ii) Conjugated with a chemotherapy drug, radioactive particle, or a toxin that poisons the targeted cells, e.g., Gemtuzumab ozogamicin for acute myeloid leukemia or tositumomab for non-Hodgkins lymphoma. Apart from being used to treat cancer, radiolabelled antibodies can also be used with special cameras to detect metastasis or spread of tumor to other organs. MoAb is given intravenously. Compared with chemotherapy, the side effects of naked MoAb are usually very mild, which include fever, chills, headache, nausea, vomiting, diarrhea, rash, and lowered blood pressure.

- Cellular therapies include treatment with natural or manipulated cells of human origin such as islet cell transplantation for the treatment of juvenile diabetes. Regenerative medicine as a new discipline has emerged from our progressive understanding of stem cell biology and our capacity to procure, grow, and differentiate embryonic stem cells. We have to await full development and differentiation of this field into specific therapies with favorable long-term outcomes.
- Dust, pollens, grass, weeds, mold spores, and danders are common allergens (see: allergy) in our environment. Food allergies to sea foods, shellfish, tree nuts, eggs, milk, wheat, soy, and their derivatives are being systematically identified and characterized for skin tests to determine potential causes of hypersensitivity or asthma. A radioallergosorbent test (RAST) uses numerous allergens (purified or semipurified) as immunosorbent.

A full discussion of each of the above categories of biological medicines and related agents covered by each of the categories is beyond the scope of this brief essay. However, ImmunoFacts is the most current and comprehensive collection of immunologic and vaccine information and is continuously updated for clinical application, including side effects [5].

Vaccines

The four traditional vaccine categories are inactivated, live attenuated, toxoid, and subunit (Fig. 4). Inactivated vaccines are created from live, virulent organisms that are killed by heat or chemicals and introduced into a host’s bloodstream to elicit an immune response against particular agents. Common examples include flu, bubonic plague, cholera, and hepatitis A. Although inactivation renders the biological harmless and diminishes the risk for infection, inactivated vaccines often produce short-lived or incomplete immunity, requiring the need for booster shots. On the other hand, live attenuated vaccines result in a more durable

Approved human vaccines	
Live vaccines	
Vaccine	Type
Smallpox	Related animal virus
BCG	Related animal bacterium attenuated
Rabies	Attenuated
Anthrax	
Yellow fever	
Oral polio vaccine	
Measles	
Mumps	
Adenovirus	
Varicella	
Rotavirus 89–12	
Rubella	
Live influenza	
Ty21a typhoid	
Influenza seed	Reassortants
Live influenza	
Rotavirus (bovine-human)	
Inactivated vaccines	
Vaccine	Type
Typhoid	Inactivated whole organism
Cholera	
Plague	
Whole-cell pertussis	
Influenza	
Inactivated polio vaccine	
hepatitis A	
Subunit vaccines	
Vaccine	Type
Japanese encephalitis	Extracts or subunits
Influenza	
Anthrax	
Cell-culture rabies	
Diphtheria	Toxoids
Tetanus	
Anthrax	
Pneumococcal	Capsular polysaccharides
Typhoid (Ty Vi)	
<i>H. influenzae</i> type b	Protein-conjugated capsular polysaccharides
Pneumococcal	
Meningococcal	
Staphylococcal	
Hepatitis B	Purified or recombinant proteins
human papillomavirus	
Acellular pertussis	
Lyme disease	
Hepatitis A/B	Combined vaccines
Tetanus/diphtheria/pertussis	

Biologicals. Figure 4 Licensed human vaccines. From Immunity: Immune Response in Inflammatory Disease by DeFranco, Locksley and Robertson [2].

immune response because the disease agent, in a sense, is only slightly virulent. Rather than an inactivated form of the organism, attenuated vaccines employ the use of the same organism grown in conditions that inhibit its ability to cause infection, usually by limiting its ability to reproduce. Common examples include measles, mumps, rubella, and yellow fever.

While the two examples discussed so far are made from whole organisms, the following two are made from their parts. A toxoid vaccine is useful when the disease-causing agent is a toxic by-product of the organism rather than the organism itself and is made by inactivating the toxin(s) and introducing them into the blood stream. The most common examples are tetanus and diphtheria. Subunit vaccines consist of other parts of the organism that may not necessarily be pathogenic (disease-causing) but are immunogenic. Usually these consist of an organism's surface proteins, such as those of HBV (manufactured in yeast) and the major capsid protein of HPV. Often these proteins are made through recombinant DNA technology that use yeast cells as protein factories and spontaneously assemble into virus-like particles (VLP).

The science of biologically derived vaccines continues to evolve. Three experimental vaccine types are noteworthy: conjugate, recombinant, and DNA vaccines. Conjugate vaccines train the immune system to react against polysaccharides in bacterial cell walls by coupling them with harmful toxins, a strategy used to confer immunity against *haemophilus influenzae* type B. Recombinant vaccines combine the physiology of one organism with the DNA of another in order to build immunity against organisms with complex infection processes. Finally, DNA vaccines have risen as the most revolutionary new vaccine for their ability to be easily manufactured and stored. Rather than challenging the immune system with antigenic proteins or polysaccharides, DNA vaccines are integrated into the host genome, where they are transcribed and translated into functional proteins. Immunity is then launched against the proteins produced within the body; in its simplicity, this embodies the same concept as production of a normal subunit vaccine but without yeast as the middleman. However, DNA vaccines are still in experimental stages with promise to be fulfilled.

Adverse effects of vaccine administration are generally mild such as those listed for MoAb and serious side effects are exceedingly uncommon. These are considerations used by regulators in approving or licensing vaccines for general use. Opposition to vaccination has existed for some time and for various reasons. Some have argued that the side effects of vaccines are not understood well enough to weigh the benefits of immunization against its costs. Some studies have suggested a link between certain vaccines and autism because of a preservative called thimerosal, which contains ethylmercury. However, thimerosal is rarely used anymore and other studies have shown little evidence for a link between vaccination and autism. In addition, health officials argue that calculating the risk/benefit ratio should be based on benefit to the community rather than to the individual.

- Targeted Cancer Therapy
- Rheumatoid Arthritis

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Biologics

► Biologics

Biomarker

A measurable DNA/RNA or protein characteristic that is an indicator of normal biological process, pathogenic process and/or response to therapeutic or other interventions, used as diagnostic and prognostic indicators.

► Gene Expression Analysis and Microarray Technology

Biopterin

(6R)-5,6,7,8-tetrahydro-L-biopterin

Biotin

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Synonyms

Hexahydro-2-oxo-1H-thieno [3,4-d] imidazol-4-valeric acid

Definition

Biotin can be synthesized by the human colon flora. The question to which extent this production contributes to covering the host-organism's requirements is, however, subject to discussion. In most foods of animal origin as well as in cereals, biotin prevails in the protein (= enzyme)-bound form as ε-N-biotinyl-L-lysine (= biocytin). Brewer's yeast, liver, soya beans, and peanuts number among the biotin rich foods [1].

The chemical structure of biotin (hexahydro-2-oxo-1H-thieno [3,4-d] imidazol-4-valeric acid) is shown in Fig. 1. Of the eight stereoisomers, only d-(+)-biotin occurs naturally and is biologically active.

Mechanism of Action

Biotin is involved in carboxylation and decarboxylation reactions. It is covalently bound to its enzyme. In the carboxylase reaction, CO₂ is first attached to biotin at the ureido nitrogen, opposite the side chain in an ATP-dependent reaction. The activated CO₂ is then transferred from carboxybiotin to the substrate. The four enzymes of the intermediary metabolism requiring biotin as a prosthetic group are pyruvate carboxylase (pyruvate ►oxaloacetate), propionyl-CoA-carboxylase (propionyl-CoA ►methylmalonyl-CoA), 3-methylcrotonyl-CoA-carboxylase (metabolism of leucine), and acetyl-CoA-carboxylase (acetyl-CoA ►malonyl-CoA) [1].

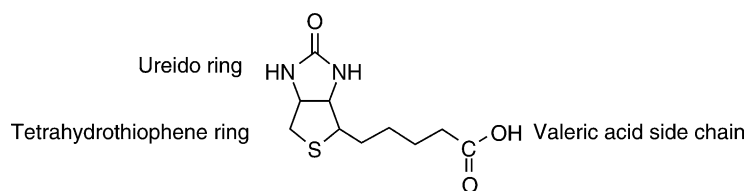
Clinical Use (Including Side Effects)

Alimentary biotin deficiency is rare. It may, however, occur in patients on long-term parenteral nutrition lacking biotin or in persons who frequently consume raw egg white. Raw egg white contains a biotin-binding glycoprotein, called avidin, which renders biotin biologically unavailable. Pharmacological doses of the vitamin (1–10 mg/d) are then used to treat deficiency symptoms. There are no reports of toxicity for daily oral doses up to 200 mg and daily intravenous doses of up to 20 mg [2].

- Folic Acid
- Niacin
- Pantothenic Acid
- Vitamin B1
- Vitamin B2
- Vitamin B6
- Vitamin B12
- Vitamin C

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Biotin. Figure 1 Structure of biotin.

BiP

Molecular chaperone (relative molecular mass 78 K) found in the lumen of the ER. BiP is related to the Hsp70 family of heat-shock proteins and was originally described as immunoglobulin heavy chain binding protein.

►Protein Trafficking and Quality Control

Bipolar Disorder

Bipolar disorder or manic depressive illness, refers to a severe mental illness characterized by recurring episodes of mania and depression.

►Antidepressant Drugs
►Galanin Receptors

Bisphosphonates

Pyrophosphate analogues that accumulate in bone and inhibit osteoclast activity. Presently, nitrogen-containing bisphosphonates such as Alendronate, Risedronate, Ibandronate or Zoledronate are being distinguished from non-aminobisphosphonates such as Clodronate or Etidronate. Bisphosphonates are among first line treatments for benign and malignant bone diseases.

►Bone Metabolism

Bistability

Technical term for properties of electrical or neural circuits (flip-flop switch) to rest in two distinct states while avoiding intermediate states (e.g., behavioral state sleep–wake transitions).

►Orexins

Bisubstrate Analogs

Bisubstrate analogs are compounds that contain features of both substrates for an enzymatic reaction in which two substrates are used.

►Lipid Modifications

BK_{Ca} Channel

Big-conductance Ca²⁺ sensitive K⁺ (BK_{Ca}) channels are activated by calcium surge and membrane depolarization. BK_{Ca} channels are specifically blocked by iberiotoxin and less selectively by charybdotoxin. BK_{Ca} channels are composed of pore-forming α and auxiliary β subunits. Both BK_{Ca, α} and BK_{Ca, β} subunits as well as their efficient coupling in the heteromultimeric formation of BK_{Ca} channel complexes are important for the function of BK_{Ca} channels.

►K⁺ Channels
►Smooth Muscle Tone Regulation
►Carbon Monoxide

Blastocyst

The blastocyst is an early embryonic stage in mammalian development. Murine blastocysts can be harvested at day 3.5 p.c. Their inner cell mass contains embryonic stem cells. Multiple murine embryonic stem cell lines have been established. Embryonic stem cells carrying genetically engineered mutations are injected into blastocysts, which are subsequently implanted into pseudopregnant foster mothers.

► Transgenic Animal Models

β -Blockers

β -blockers are antagonists of β -adrenergic receptors.

► β -Adrenergic System

Blood–brain Barrier

The blood–brain barrier (BBB) forms a physiological barrier between the central nervous system and the blood circulation. It consists of glial cells and a special species of endothelial cells, which form tight junctions between each other; thereby inhibiting paracellular transport. In addition, the endothelial cells of the BBB express a variety of ABC-transporters to protect the brain tissue against toxic metabolites and xenobiotics. The BBB is permeable to water, glucose, sodium chloride and non-ionised lipid-soluble molecules but large molecules such as peptides as well as many polar substances do not readily permeate the barrier.

- ABC-Transporter
- Sleep
- Emesis

Blood Clotting

► Coagulation/Thrombosis

Blood Pressure Control

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Synonyms

Physiology of blood pressure; Control of hypertension

Definition

The blood pressure represents the tension or pressure of the blood within the arteries that is exerted against the arterial wall in vivo. Blood pressure is a quantitative trait that is highly variable. In population studies, blood pressure has a normal distribution that is slightly skewed to the right. The regulation of blood pressure within the intravascular system is a complex interaction of a number of systems and mechanisms. A chronic elevation of blood pressure or hypertension is a substantial health problem affecting 25% of the adult population in industrialized societies. Despite important advances in our understanding of the pathophysiology of hypertension and the availability of effective treatment strategies it still remains a major modifiable risk factor for cardiovascular and renal disease. There is no specific level of blood pressure where clinical complications start to occur; thus the definition of hypertension is arbitrary but needed in clinical practice for patient assessment and treatment.

The relevance of clinical conditions with chronic low blood pressure or hypotension has been questioned, with the exception of a few rare clinical syndromes. Temporary increases or decreases of blood pressure are often seen in clinical medicine in the context of acute illnesses or interventions.

Basic Mechanisms

The circulation is divided into several compartments: the high-pressure arterial circuit, which contains 13% of the blood volume, the capillary bed containing 7% of the blood volume, and the low-pressure venous bed, which contains 64% of the blood volume. The pulmonary circulation contains 9% and the heart 7% of the blood volume. Although the venous system stores and propels large volumes of blood and regulates cardiac output by venous return to the heart, in considering blood pressure control attention is focused on the high-pressure arteries. The basic function of the circulation is to provide nutrients to peripheral tissues. Blood vessels in local tissue beds regulate blood flow in relation to local needs. Blood flow (Q) is defined by Ohm's law and varies directly with the change in pressure (P) across a blood vessel and

inversely with the resistance R ($Q = P/R$). It can be seen that pressure varies directly with blood flow and resistance ($P = QR$). Blood pressure is produced by the contraction of the left ventricle (producing blood flow) and by the resistance of the arteries and arterioles. Systolic pressure, or maximum blood pressure, occurs during left ventricular systole. Diastolic pressure, or minimum blood pressure, occurs during ventricular diastole. The difference between systolic and diastolic pressure is the pulse pressure (►systolic and diastolic blood pressure and pulse pressure).

Although blood pressure control follows Ohm's law and seems to be simple, it underlies a complex circuit of interrelated systems. Hence, numerous physiologic systems that have pleiotropic effects and interact in complex fashion have been found to modulate blood pressure. Because of their number and complexity it is beyond the scope of the current account to cover all mechanisms and feedback circuits involved in blood pressure control. Rather, an overview of the clinically most relevant ones is presented. These systems include the heart, the blood vessels, the extracellular volume, the kidneys, the nervous system, a variety of humoral factors, and molecular events at the cellular level. They are intertwined to maintain adequate tissue perfusion and nutrition. Normal blood pressure control can be related to cardiac output and the total peripheral resistance. The stroke volume and the heart rate determine cardiac output. Each cycle of cardiac contraction propels a bolus of about 70 ml blood into the systemic arterial system. As one example of the interaction of these multiple systems, the stroke volume is dependent in part on intravascular volume regulated by the kidneys as well as on myocardial contractility. The latter is, in turn, a complex function involving sympathetic and parasympathetic control of heart rate; intrinsic activity of the cardiac conduction system; complex membrane transport and cellular events requiring influx of calcium, which lead to myocardial fibre shortening and relaxation; and affects the humoral substances (e.g., catecholamines) in stimulation heart rate and myocardial fibre tension.

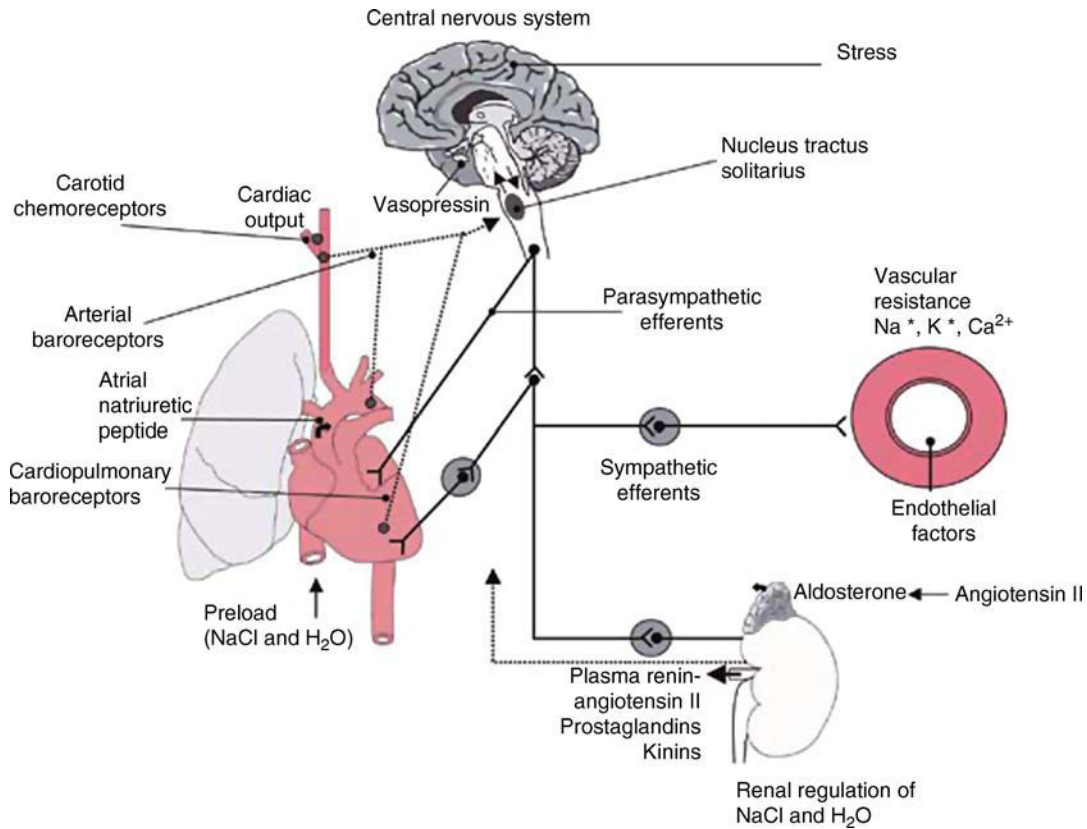
The regulation of the total peripheral resistance also involves the complex interactions of several mechanisms. These include baroreflexes and sympathetic nervous system activity; response to neurohumoral substances and endothelial factors; myogenic adjustments at the cellular level, some mediated by ion channels and events at the cellular membrane; and intercellular events mediated by receptors and mechanisms for signal transduction. As examples of some of these mechanisms, there are two major neural reflex arcs (Fig. 1). Baroreflexes are derived from high-pressure baroreceptors in the aortic arch and carotid sinus and low-pressure cardiopulmonary baroreceptors in ventricles and atria. These receptors respond to stretch (high pressure) or

filling pressures (low pressure) and send tonic inhibitory signals to the brainstem (nucleus tractus solitarius). If blood pressure increases and tonic inhibition increases, inhibition of sympathetic efferent outflow occurs and decreases vascular resistance and heart rate. If blood pressure decreases, however, less tonic inhibition ensues from the baroreflexes and both heart rate and peripheral vascular resistance increase, thereby increasing blood pressure. In addition, the neural control of renal function produces alterations in renal blood flow; glomerular filtration rate (GFR); excretion of sodium, other ions, and water; and release of renin and other vasoactive substances. These, in turn, have effects on the regulation of intravascular volume, vascular resistance and blood pressure. Activation of carotid chemoreceptors is also transmitted to the vasomotor centre and responds not only to arterial pressure but also to oxygen tension and carbon dioxide tension (in opposite directions). A drop in blood pressure, a drop in oxygen tension, or a rise in dioxide tension results in increased sympathetic outflow to the adrenal medulla, heart, and resistance vessels.

Additional cellular events linked to the activity of blood pressure regulating substances involve membrane sodium transport mechanisms; Na^+/K^+ ATPase; Na^+/Li^+ countertransport; Na^+-H^+ exchange; $\text{Na}^+-\text{Ca}^{2+}$ exchange; Na^+-K^+ 2Cl transport; passive Na^+ transport; potassium channels; cell volume and intracellular pH changes; and calcium channels.

Numerous vasoactive substances have major effects on blood vessels, the heart, the kidneys, and the central nervous system (CNS) and often serve to counterbalance one another. As examples of physiologic actions, norepinephrine (noradrenaline), via α -adrenergic mechanisms, is a potent vasoconstrictor, while epinephrine (adrenaline), via α - and β -adrenoceptors, increases primarily heart rate, stroke volume, systolic blood pressure and pulse pressure. The ►renin–angiotensin aldosterone system generates angiotensin II (Ang II). Ang II, in turn, constricts vascular smooth muscle, stimulates aldosterone secretion, potentiates sympathetic nervous system activity, leads to salt and water reabsorption in the proximal tubule, stimulates prostaglandin, nitric oxide, and endothelin release, increases thirst, and is a growth factor. Aldosterone activates the epithelial sodium channel (ENaC) in the cortical collecting duct in the kidney, leading to sodium reabsorption and potassium excretion. Prostaglandin E and prostacyclin act to counterbalance vasoconstriction by Ang II and norepinephrine.

Vasopressin (antidiuretic hormone [ADH]) secretion increases in response to decreased blood volume and/or reductions in effective blood volume via a decrease in inhibitory tone from both low-pressure and high-pressure baroreceptors to the hypothalamus. The neuronal pathways that mediate hemodynamic regulation of



Blood Pressure Control. Figure 1 Basic mechanisms involved blood pressure control. The most important organs involved in blood pressure control are shown (for explanations see text). In these organs a variety of different cell types and molecular events are related to blood pressure control. Physiological effects are initiated by a wide spectrum of vasoactive substances: (a) catecholamines such as norepinephrine and epinephrine bind to different adrenergic receptors (α_1 , α_2 , β_1 , β_2) causing protein phosphorylation and increased intracellular calcium via G-protein-coupled receptors linked to ion channels or second messengers (cyclic nucleotides, phosphoinositide hydrolysis). (b) Ang II as the effector peptide of the RAS binds to angiotensin (AT_1 , AT_2 and others)-receptors causing increased intracellular calcium and protein phosphorylation via second messenger, phosphoinositide hydrolysis, and activated protein kinases, and aldosterone secretion. (c) Endothelial derived factors such as nitric oxide (NO) increase levels of cGMP and activation of protein kinases, while endothelins (most importantly endothelin-1 [ET-1]) activate G-proteins, phospholipase C and L-type calcium channels. (d) The effects of atrial natriuretic peptide (ANP) and of brain and C-type natriuretic peptides are mediated via cGMP upon stimulation of three different receptors.

vasopressin release are completely different from those involved in osmoregulation and unlike the latter, small decreases in blood pressure or blood volume have little effect on vasopressin secretion. A rise in blood pressure causes a decrease in secretion of vasopressin related to increased baroreceptor activity, which inhibits hypothalamic vasopressin-releasing hormones. Vasopressin works by causing water conservation at the distal collecting duct of nephron. This alone, however, is a relatively inefficient mechanism of increasing intravascular volume because conserved water is distributed among total body water and only a small portion is intravascular. In addition, vasopressin is a potent vasoconstrictor and the greater vasopressin secretion observed in response to more severe hypovolemia or hypotension serves as a mechanism to stave off cardiovascular collapse

during periods of large reductions of blood pressure or blood volume. Two important endothelial derived factors have opposite effects on the blood vessels: nitric oxide is a vasodilator whereas the endothelins, particularly endothelin-1, are vasoconstrictors. The kallikrein-kinin system produces vasodilator kinins, which in turn may stimulate prostaglandins and nitric oxide. Natriuretic peptides induce vasodilation, induce natriuresis, and inhibit other vasoconstrictors (renin-angiotensin, sympathetic nervous system and endothelin).

When the temporal sequence of adjustments of blood pressure is analysed it seems, that CNS mechanisms (e.g., baroreflexes) will provide regulation of the circulation within seconds to minutes. Other mechanisms, such as the renin-angiotensin-aldosterone system and fluid shifts, occur over minutes to hours. Only the

kidneys seem to have the ability for long-term adjustment in blood pressure, predominantly through regulation of extracellular volume. This theoretical concept has recently been – although indirectly – confirmed by genetic approaches applied to the analysis of rare familial syndromes of high blood pressure, i.e., hypertension, or low blood pressure, i.e., hypotension. In those studies carried out in families with monogenic forms of the disease several molecular pathways have been successfully delineated. All defects identified so far raise or lower blood pressure through a common pathway by increasing or decreasing salt and water reabsorption by the nephron. Thus, these studies point to the kidney as a (the) pivotal organ for chronic (genetic) determination of blood pressure.

Pharmacological Intervention

The morbid consequences of high blood pressure have been documented by epidemiologic studies, which demonstrate a strong positive and continuous correlation between blood pressure and the risk of cardiovascular disease (stroke, myocardial infarction, heart failure), renal disease and mortality. This correlation is more robust with systolic than with diastolic blood pressure. While hypertension was once thought to be “essential” for perfusion of tissues through sclerotic and narrowed blood vessels and to maintain a normal sodium balance, the pathologic nature of elevated blood pressure has become clear. Essential, primary, or idiopathic hypertension is defined as high blood pressure in which causes such as renovascular disease, renal failure, pheochromocytoma, aldosteronism, or other causes of secondary hypertension or monogenic (mendelian) forms are not present. Essential hypertension accounts for 95% of all cases of hypertension. This condition is a heterogeneous disorder, with different patients having different causal factors that lead to high blood pressure. There are no established clinical or laboratory tests to identify the factors that are responsible for the blood pressure elevation in an

individual patient. Consequently, pharmacologic treatment of essential hypertension is largely empiric. Clinical treatment algorithms try to account for comorbidities and expected or observed side effects of drugs in individual patients. Pharmacologic treatment should always be implemented “on top” of nonpharmacologic interventions such as control of body weight, alcohol intake, salt intake and modifications of lifestyle as necessary in the individual patient. Randomised trial of pharmacologic treatment of hypertension have documented that blood pressure reduction lowers morbidity and mortality, with dramatic reduction in stroke and smaller reductions in cardiac and renal disease (for details see ►[antihypertensive drugs](#)).

As a result of such studies hypertension has been operationally defined as the blood pressure level above which therapeutic intervention has clinical benefit. As increasingly aggressive intervention has continued to demonstrate benefits, this level has gradually reduced over time and is commonly defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg (Table 1). Isolated systolic hypertension is defined as systolic blood pressure ≥ 140 mmHg and diastolic blood pressure < 90 mmHg.

Moreover, in patients with certain comorbidities, such as diabetes mellitus, left ventricular dysfunction or chronic nephropathy, lower blood pressures levels in the range of 130/80 mmHg (125/75 mmHg for nephropathies with overt proteinuria > 1 g per day) are currently established as target blood pressure. Individuals with high normal blood pressure tend to maintain pressure that are above average for the general population and are at greater risk for development of definite hypertension and cardiovascular events than the general population. These observations leave open the question of whether there is an “optimal” blood pressure in the general population, and whether small reductions in blood pressure across the entire population, rather than larger targeted reductions, would have benefit.

Blood Pressure Control. Table 1 Definitions and classifications of blood pressure levels

Category	Systolic [mm Hg]		Diastolic [mm Hg]	Stroke mortality relative risk
Optimal	< 120	And	< 80	1.00
Normal	< 130	And	< 85	1.73
High normal	130–139	Or	85–90	2.14
Hypertension				
Stage 1 (mild)	140–149	Or	90–99	3.58
Subgroup: borderline	140–159	Or	90–94	
Stage 2 (moderate)	160–179	Or	100–109	6.90
Stage 3 (severe)	≥ 180	Or	≥ 110	9.66
Isolated systolic hypertension	≥ 140	And	< 90	
Subgroup: borderline	140–149	And	< 90	

The higher category applies, if systolic and diastolic blood pressure values of a patient fall into different categories.

Nonetheless, in patients with established hypertension according to the above criteria, antihypertensive drug treatment is one of the most important means to prevent or reduce overall cardiovascular and renal disease. There is hope that the information gained from the Human Genome Project and from ongoing research aimed at dissecting the genetic basis of hypertension in both human populations and animal models will foster the development of more effective and targeted, i.e., pharmacogenetic, treatments based on the genotype of the individual patient.

- ▶ Antihypertensive Drugs
- ▶ Renin–Angiotensin–Aldosterone System
- ▶ Smooth Muscle Tone Regulation

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Blood Pressure Lowering Drugs

- ▶ Antihypertensive Drugs

Blood Products

- ▶ Biologicals

BMPs

Bone Morphogenetic Proteins

Body Mass Index (BMI)

Anthropometric measure of body mass that is calculated by dividing a person's weight in kilograms by the square of their height in metres.

- ▶ Orexins
- ▶ Anti-obesity Drugs

Bombesin-like Peptides

Bombesin-like peptides are a group of biologically active peptides, which are structurally related, including bombesin (a tetradecapeptide originally derived from amphibians), gastrin-releasing peptide (GRP), neuromedin B (NMB), and GRP_{18–27} (previously named “neuromedin C”). Bombesin and bombesin-like peptides have a wide range of functions. They have been shown to promote cell growth, including that of carcinoma cell lines, they are involved in smooth muscle contraction and in the regulation of the body temperature. Bombesin/bombesin-like peptides also mediate endocrine responses, such as the release of gastrin, cholecystokinin, and pancreatic polypeptide. This group of peptides has been involved in the regulation of various behavioral responses, the regulation of pain and the modulation of feeding behavior. Bombesin/bombesin-like peptides exert their effects through a group of G-protein coupled receptors, which signal through G_{q/11} (BB1, BB2, BB3). While BB1 responds mainly to NMB and bombesin, BB2 responds to GRP and bombesin.

Bone Metabolism

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Synonyms

Bone turnover; Skeletal turnover; Skeletal metabolism; Bone remodelling

Definition

Bone metabolism comprises the processes of bone formation and bone resorption, the key actions by which skeletal mass, structure and quality are accrued and maintained throughout life. In the mature skeleton, anabolic and catabolic actions are mostly balanced due to the tight regulation of the activity of bone forming (►osteoblast) and bone resorbing (►osteoclast) cells through circulating ►osteotropic hormones and locally active cytokines.

Basic Mechanisms

Biochemical Composition of Bone

Bone is composed of approximately 70% mineral (mainly hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ crystals) and 30% organic matter (i.e. cells, collagen and non-collagenous proteins). Most of the latter is synthesised by local osteoblasts. Ninety per cent of the organic extracellular matrix of bone consists of type I collagen, which assembles into fibrils and is then covalently cross-linked to generate tensile strength. Of the non-collagenous proteins, osteocalcin, bone sialoprotein and osteopontin are the most abundant. Most of the non-collagenous proteins play important roles in the organisation of the extracellular matrix (cell–cell and cell–matrix attachment, cell migration, growth, development, fibril formation). Besides these components, several specific enzymes and small peptides are an integral part of the organic matrix and play an important role in skeletal metabolism (see below). The calcium–collagen–cell composite ensures the two main functions of bone: providing a structural framework and a reservoir for mineral ions.

Bone Structure

Bone is a complex structure consisting of diverse cell types, extracellular matrix and mineral. Bone matrix is composed predominantly of extensively cross-linked collagen type I but also incorporates many other proteins including osteocalcin, osteopontin, proteoglycans and growth factors. The mineral phase is calcium phosphate in the form of hydroxyapatite. This mineral component provides mechanical strength and rigidity to the skeleton and is sufficiently labile to be easily removed, enabling it to act as an ion store for supporting calcium homeostasis.

Most bones of the human skeleton are composed of two structurally distinct types of tissue: compact (dense) and trabecular (cancellous, spongy) bone. Both types contain the same elements: cells (►osteocytes) embedded in a mineralised matrix and connected by small canals (“canaliculi”). In compact bone, which makes up 85% of the skeleton, these components form elongated cylinders of concentric lamellae surrounding a central blood vessel (called osteon or Haversian system). Cancellous bone, in contrast, forms thin,

interconnected spicules. Compact bone constitutes the outer parts of many bones (“compacta”), shows a high degree of mineralisation, a comparatively low number of cells, and provides the framework for the interior cancellous parts of bone. The latter consists of a sponge-like network of small beams (trabeculae) with a larger number of cells (osteoblasts and osteoclasts) distributed over a huge surface.

Bone is under continual stress and strain as it supports the body and provides the rigid structure required for weight bearing and movement. As a result, over time, it develops fatigue damage in the form of cracks and tiny fractures, which must be repaired to maintain skeletal integrity. Bone also must be reshaped during growth and fracture healing. To shape and repair bone, and to release calcium in times of dietary calcium deficiency, the body must be able to remove, replace and increase amounts of bone as required. This ability is achieved by the coupled processes of bone formation and bone resorption, which are continuous throughout life, resulting in renewal of the entire skeleton every 7–10 years. The most important cells involved in these processes are the bone-resorbing osteoclasts, the bone-forming osteoblasts and the osteoblast-related bone lining cells and osteocytes.

Osteoclasts and Bone Resorption

Bone is resorbed by highly specialised cells called osteoclasts. Osteoclasts are related to macrophages and develop from a common monocyte precursor. Osteoclasts remove bone by adhering to bone and forming a sealed cavity against the bone surface. Along this cavity, osteoclasts then develop a specialised membranous structure, the ‘ruffled border’, through which acid (H^+) and proteases are secreted to dissolve both the mineral and matrix components of bone, creating a resorption pit also known as Howship’s lacuna. The minerals and protein fragments released during this process are absorbed into the osteoclast by endocytosis, partly processed and later secreted from the distal side of the osteoclast, from where they eventually reach the circulation.

Osteoclast recruitment and activity are each highly regulated by cells of the osteoblast lineage. Osteocytes detect strain and microdamage in bone and signal the requirement for bone resorption and repair. Osteoblasts respond to a multitude of signals, including ►parathyroid hormone (PTH) and ►vitamin D, as well paracrine signals on a more local level. Osteoblasts integrate these signals and induce bone resorption by increasing their secretion of a cytokine known as receptor activator of nuclear factor kappa (RANK) ligand (RANKL), and by reducing expression of osteoprotegerin (OPG), a natural antagonist of RANKL. RANKL acts by binding to its receptor, RANK, on osteoclast precursors to induce these cells to differentiate into osteoclasts and

also acts on existing osteoclasts to increase their bone resorbing activity. When the requirement for bone resorption is completed, the osteoblasts increase their secretion of OPG, which binds to RANKL preventing its interaction with its receptor on osteoclasts resulting in the inactivation and death of osteoclasts (Fig. 1).

Bone Formation

Osteoblasts are the primary cells responsible for bone formation. They are derived from mesenchymal (stromal) cells that first differentiate into pre-osteoblasts and then into mature, bone matrix producing osteoblasts. Inactivated or “resting” osteoblasts become “lining cells” and thus a reservoir for bone forming cells to be activated at the next remodelling cycle. Osteoblasts trapped and embedded in the mineralised matrix are called osteocytes, and are important for many properties of living bone.

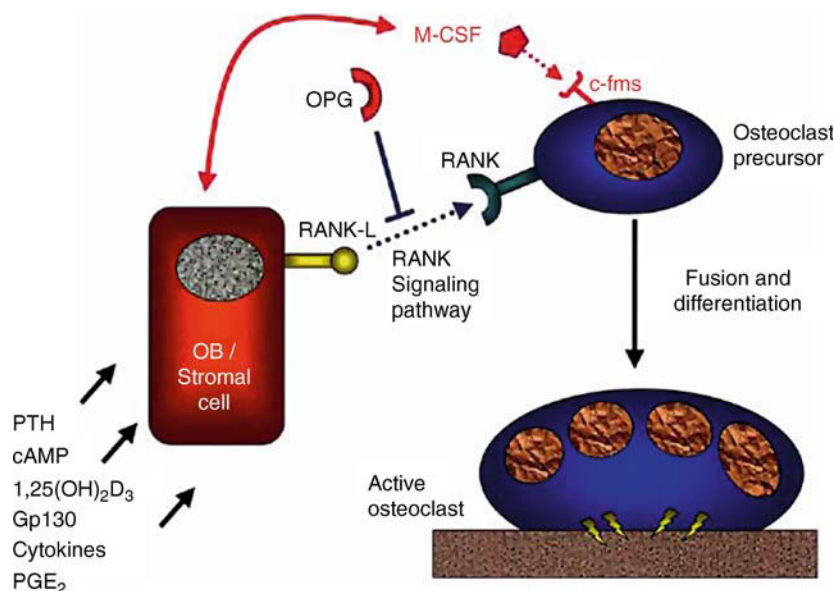
During bone formation, a series of sequential changes occur in cells in the osteoblast lineage, including osteoblast chemotaxis, proliferation and differentiation, which in turn is followed by formation of mineralised bone and cessation of osteoblast activity. The osteoblast changes are preceded by osteoclast apoptosis, which may be dependent on active TGF- β released from the resorbed bone. This is followed by chemotactic attraction of osteoblasts or their precursors to the sites of the resorption defect. Chemotactic attraction of osteoblast precursors is likely mediated by local factors produced during the resorption process.

Proliferation of osteoblast precursors is an important event at the remodeling site. This is also likely to be enhanced by local osteoblast growth factors released

during the resorption process. Likely candidates include members of the TGF- β superfamily, such as platelet derived growth factor (PDGF), insulin-like growth factors (IGF) and the fibroblast growth factors (FGFs). All of these substances are stored in the bone matrix.

The next sequential event during the formation phase is the differentiation of the osteoblast precursor into the mature cell. Several of the bone-derived growth factors can cause the appearance of markers of the differentiated osteoblast phenotype, including expression of alkaline phosphatase activity, Type I collagen and osteocalcin synthesis. Most prominent of these are bone morphogenetic protein 2 (BMP-2) and IGF-I. Recent investigations demonstrate that ‘Wnt’ signalling plays a critical role in controlling osteoblast differentiation. Wnts form a dual-receptor complex with frizzled and low-density-lipoprotein (LDL)-receptor like protein 5 (LRP5) on cell surfaces. The role of LRP5 in bone mineral density (BMD) determination was initially uncovered by genetic analyses that mapped the osteoporosis pseudoglioma (OPPG) syndrome and the high bone mass (HBM) syndrome to the human chromosome 11q12–13 locus where LRP5 was identified. Circulating hormones such as PTH and PTH-related peptide (PTHrP), 1,25-dihydroxyvitamin D₃ are also important stimulators of osteoblast differentiation.

The final phase of the formation process is cessation of osteoblast activity. The resorption lacunae are usually repaired completely. It is not known how this level of time regulation is achieved. One possibility is that factors produced during osteoblast differentiation decrease osteoblast activity. One such factor could be TGF- β since active TGF- β decreases differentiated function in osteoblasts.



Bone Metabolism. Figure 1 Mechanisms of osteoclast activation.

Bone Remodelling

Bone remodelling, which continues throughout adult life, is necessary for the maintenance of normal bone structure and requires that bone formation and resorption should be balanced. Bone remodelling occurs in focal or discrete packets known as bone multicellular unit (BMU). In this process, both bone formation and resorption occur at the same place so that there is no change in the shape of the bone. After a certain amount of bone is removed as a result of osteoclastic resorption and the osteoclasts have moved away from the site, a reversal phase takes place in which a cement line is laid down. Osteoblasts then synthesize matrix, which becomes mineralised. The BMU remodeling sequence normally takes about 3 months to produce a bone structure unit (Fig. 2).

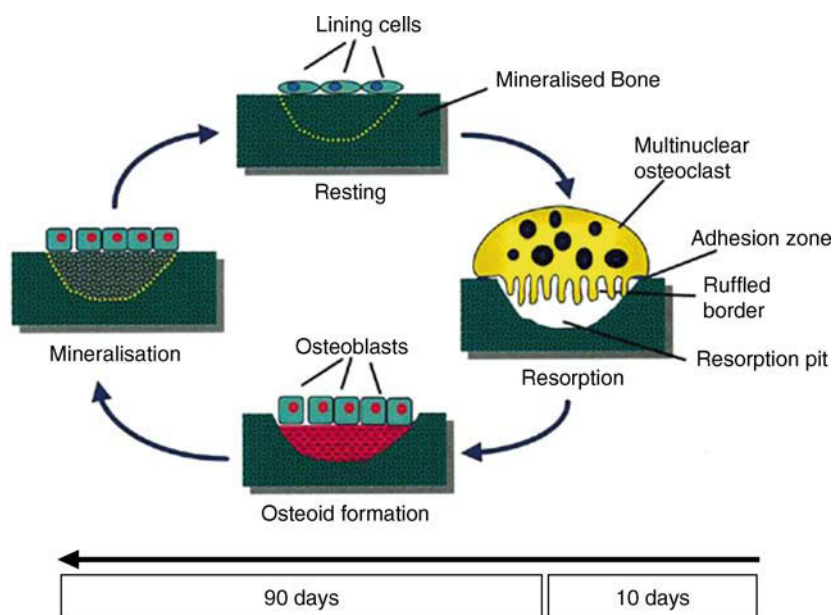
The constant remodeling of bone is important in three ways: First, bone remodeling is needed to repair fatigue damage. Bone, like other structural materials that undergo repetitive cyclical loadings, are subject to fatigue. After a number of loading cycles, tiny cracks may form. If these tiny cracks are not fixed in a timely fashion, they will accumulate and eventually lead to structural failures. Bone remodeling will replace bone-containing cracks and prevent structural failure. Secondly, bone remodeling is needed to adapt bone material properties to the mechanical demands that are placed on bones. For instance, bone remodeling will help you obtain stronger bone with exercise. Finally, bone remodeling plays a critical role in regulating calcium homeostasis, which is critical for life. Bone is a major reservoir for calcium. When serum calcium levels

fall and there is no dietary calcium intake, calcium will be mobilised from bone by osteoclasts to meet the demand.

Hormonal Regulation of Calcium Homeostasis and Bone Remodelling

PTH is the most important regulator of bone remodeling and calcium homeostasis. PTH is an 84-amino acid polypeptide and is secreted by the parathyroid glands in response to reductions in blood levels of ionised calcium. The primary physiological effect of PTH is to increase serum calcium. To this aim, PTH acts on the kidney to decrease urine calcium, increase urine phosphate, and increase the conversion of 25-OH-vitamin D to 1,25-(OH)₂-vitamin D. PTH acts on bone acutely to increase bone resorption and thus release skeletal calcium into the circulation. However, due to the coupling of bone resorption and bone formation, the longer-term effect of increased PTH secretion is to increase both bone resorption and bone formation.

The steroid hormone 1,25-dihydroxyvitamin D₃ (calcitriol) slowly increases both intestinal calcium absorption and bone resorption, and is also stimulated through low calcium levels. In contrast, **calcitonin** rapidly inhibits osteoclast activity and thus decreases serum calcium levels. Calcitonin is secreted by the clear cells of the thyroid and inhibits osteoclast activity by increasing the intracellular cyclic AMP content via binding to a specific cell surface receptor, thus causing a contraction of the resorbing cell membrane. The biological relevance of calcitonin in human calcium homeostasis is not well established.



Bone Metabolism. Figure 2 The bone remodelling cycle.

Pharmacological Interventions

In the past decade, a number of novel and effective pharmacological strategies have been developed to modulate cell differentiation and/or activity in metabolic bone diseases such as osteoporosis or Paget's disease of bone, and an even greater number of potential drugs are being explored (Table 1). Agents that inhibit osteoclast formation and/or activity are usually classified as anti-resorptive or anti-catabolic drugs, while stimulators of osteoblast formation and/or activity are called "anabolic" agents. Some compounds, such as vitamin D, strontium ranelate or oestrogens seem to affect both processes.

Oestrogens reduce bone loss by inhibiting the generation of new osteoclasts, reducing the activation frequency and promoting apoptosis of mature osteoclasts via mechanisms not well understood. Some of the effects of oestrogen seem to be mediated via the modulation of growth factors and cytokines, while others are associated with binding to at least two different oestrogen receptors (ER α , ER β). Oestrogens have been shown to reduce the risk of osteoporotic fracture (and of colon cancer), but carry a (small) risk of breast cancer and cardiovascular events. Currently, their long-term use to prevent osteoporotic fractures is not being recommended.

The presence of different ERs is also, among other facts, the basis for the development of ►*selective oestrogen receptor modulators* (SERMs). While Tamoxifene, a triphenylethylene compound used in the treatment of breast cancer, has long been known to moderately inhibit bone loss, newer synthetic compounds (such as raloxifene) have been shown to reduce fracture risk in postmenopausal women. The mechanism of action is probably the same as the one suspected in oestrogens, i.e. the inhibition of cytokines causing osteoclast recruitment and differentiation. Oestrogens and SERMs are not potent enough to inhibit the grossly exaggerated osteoclast activity in malignant and Paget's disease of bone.

Due to its anti-resorptive effects, calcitonin has been widely used to treat a variety of metabolic bone diseases, including osteoporosis. However, the availability of more potent drugs has lead to a decline in the clinical use of calcitonin.

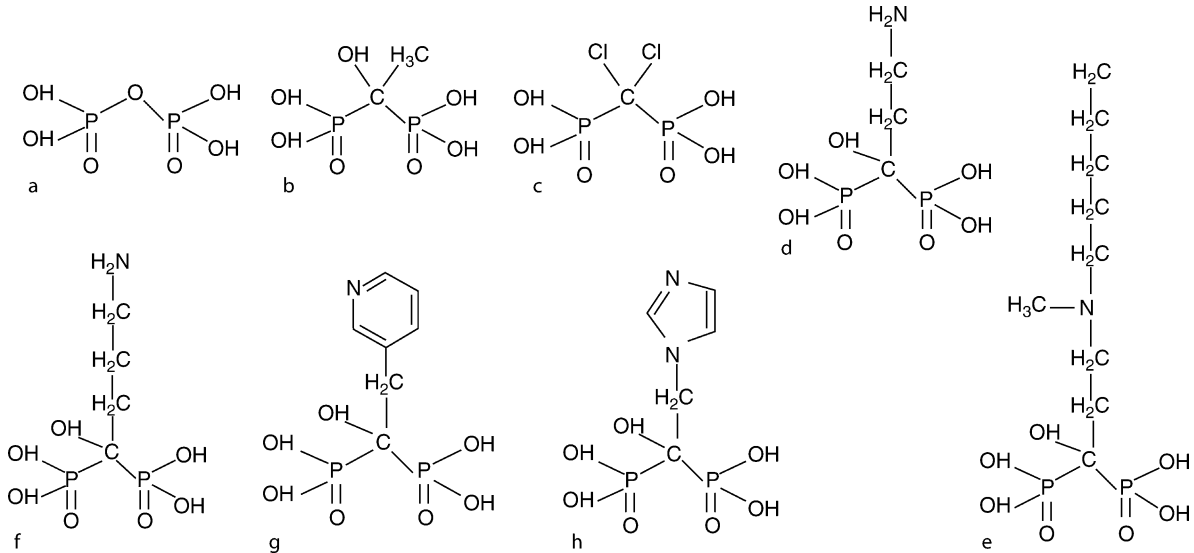
Bisphosphonates (BP) are today the first line treatment of benign and malignant bone diseases. As pyrophosphate analogues (Fig. 3), BP accumulate in bone and are taken up by osteoclasts. Once in the cell, the nitrogen-containing BP (N-BP) such as Alendronate, Risedronate, Ibandronate and Zoledronate effectively inhibit osteoclast resorption and induce cell

Bone Metabolism. Table 1 Current and future agents to modulate bone metabolism

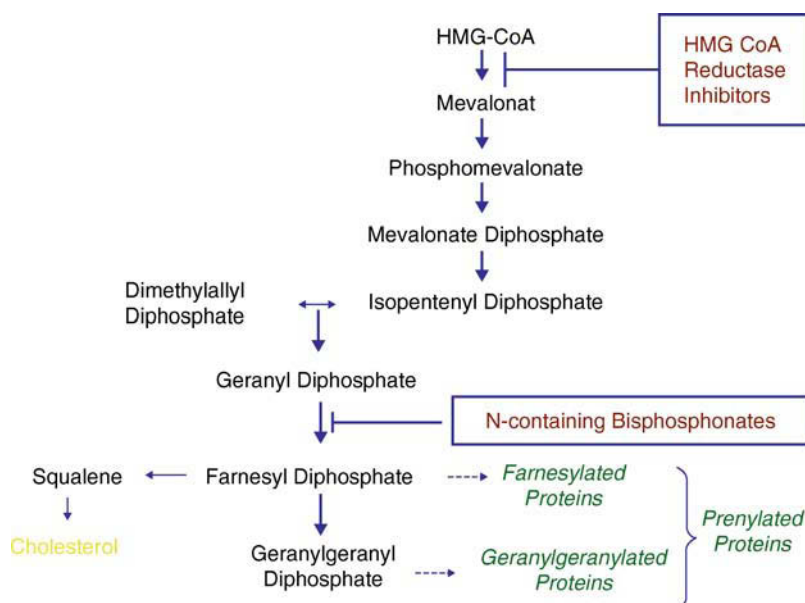
Inhibitors of osteoclast activity	Stimulators of osteoblast activity
<i>Currently in use</i>	
Bisphosphonates	Parathyroid hormone,
Selective oestrogen receptor modulators (SERMs, e.g. Raloxifene)	Calcitriol and analogues (limited use)
	Androgens (limited use)
Strontium salts (Sr Ranelate)	Fluorides (limited use)
Vitamin D	
Vitamin D metabolites and analogues	
Testosterone (limited use)	
Oestrogen (limited use)	
Calcitonin and synthetic analogues (limited use)	
<i>Under Investigation</i>	
Statins	PTH-rP and analogues
Calcium receptor modulators	High dose oestrogens
Osteoprotegerin	Statins
Denusomab (anti-RANKL mAb)	Growth factors (e.g. GH, IGFs, FGFs)
Anti-cytokines (e.g. IL-1, TNFs, IL-1 receptor constructs)	Prostaglandins and PG mimetics
	Endothelins and analogues
Proton pump inhibitors	Amylin and analogues
Nitric oxide modulators	Mechanoreceptor modulators, e.g. Glutamate
Enzyme inhibitors (e.g. metalloproteinases, cathepsin K)	Purinergic modulators
	Proteasome inhibitors
Adhesion molecule inhibitors	Intracellular signalling targets (eg SMADs)

apoptosis by inhibiting farnesyl diphosphate synthetase, an enzyme of the mevalonate pathway (Fig. 4). This in turn leads to a reduction in geranylgeranyl diphosphate and in the prenylation of GTP-binding proteins essential to osteoclast organisation and survival. Non-aminobisphosphonates such as Clodronate or Etidronate are incorporated into intracellular analogues of ATP and cause cell death.

Bisphosphonates have been shown to be highly effective in osteoporosis, cancer bone metastasis, multiple myeloma, and Paget's disease of bone. While generally very well tolerated, these drugs do have potential adverse effects. Recently, the association of long-term high dose bisphosphonate treatment with osteonecrosis of the jaw has been described. This is a potentially serious side effect seen mostly in patients with multiple myeloma or



Bone Metabolism. Figure 3 Chemical structure of pyrophosphate (a) and the bisphosphonates etidronate (b), clodronate (c), pamidronate (d), ibandronate (e), alendronate (f), risedronate (g), zoledronate (h).



Bone Metabolism. Figure 4 The mevalonate pathway. Interferences by statins and N-containing bisphosphonates are shown.

breast cancer bone metastases who receive intravenous bisphosphonate treatment. While the aetiology is uncertain, a strong association with dental pathology and interventions highlights the need for close attention to dental health in this patient group.

OPG has been shown to reduce bone turnover in postmenopausal women. More recently, Denosumab, an anti-RANKL mAb, has been tested for its ability to increase BMD and to reduce bone turnover. Results were promising and clinical phase III studies with fracture endpoints are presently under way.

Statins lower plasma cholesterol levels by inhibiting HMG-CoA reductase in the mevalonate pathway (Fig. 4). Some research has shown that certain statins (but not all) stimulate BMP-2 expression in osteoblasts, increase bone formation and mimic N-BP in that they inhibit bone resorption. The use of statins in osteoporosis is presently being investigated.

Fluorid ions stimulate bone formation by a direct mitogenic effect on osteoblasts mediated via protein kinase activation and other pathways. Further to these cellular effects, **fluorides** alter hydroxyapatite crystals in the bone matrix. In low doses, fluorides induce lamellar bone, while at higher doses abnormal woven bone with inferior quality is formed. The effect of fluorides on normal and abnormal (e.g. osteoporotic) bone therefore depends on the dose administered.

PTH has a dual effect on bone cells, depending on the temporal mode of administration: given intermittently, PTH stimulates osteoblast activity and leads to substantial increases in bone density. In contrast, when given (or secreted) continuously, PTH stimulates osteoclast-mediated bone resorption and suppresses osteoblast activity. Further to its direct effects on bone cells, PTH also enhances renal calcium re-absorption and phosphate clearance, as well as renal synthesis of 1,25-dihydroxyvitamin D. Both PTH and 1,25-dihydroxyvitamin D act synergistically on bone to increase serum calcium levels and are closely involved in the regulation of the calcium/phosphate balance. The anabolic effects of PTH on osteoblasts are probably both direct and indirect via growth factors such as IGF-1 and TGF β . The multiple signal transduction

pathways mediating the effects of PTH on bone cells include activation of cyclic AMP, intracellular protein phosphorylation, activation of phospholipase C, protein kinase C, tyrosine kinase c-src, and the generation of inositol 1,4,5-triphosphate (IP3). Teriparatide (1–34 PTH) has been shown to be highly effective in reducing the risk of osteoporotic fractures. The drug is given daily in a subcutaneous dose.

Oral calcium has long been used for the treatment of osteoporosis, both in the form of dietary and pharmacological supplements. In patients with calcium deficiency, oral calcium at doses of 1000–1500 mg/day corrects a negative calcium balance and suppresses PTH secretion. Sufficient calcium intake is most important for the accrual of peak bone mass in the young, but is also considered the basis of most anti-osteoporotic regimens. In the elderly, supplementation with oral calcium and vitamin D reduces the risk of hip fracture by about 30–40%.

Vitamin D and its metabolites are also widely used for the treatment of osteoporosis. Vitamin D₃, or cholecalciferol, is synthesised in the skin through UV radiation (sun exposure) and is the main source of vitamin D in humans. Vitamin D₂, or ergocalciferol, is derived from the diet and plays a minor role in human vitamin D supplies. Both forms are metabolised in the liver to 25-hydroxyvitamin D (calcidiol). The latter is the storage form of vitamin D and is believed to have little biologic activity. The active form of Vitamin D is generated in the kidney through the hydroxylation of C1, leading to 1,25-dihydroxyvitamin D (calcitriol). This last step is tightly controlled by a number of regulators such as PTH or serum phosphate levels. 1,25-Dihydroxyvitamin D is a potent steroid hormone with almost countless effects throughout the body. Most of these effects concern the differentiation of immature cells. As regards bone, 1,25-dihydroxyvitamin D has differentiation-inducing as well as activating effects on both osteoblasts and osteoclasts. It also increases the absorption of calcium from the gut. Data from excellent clinical trials show that the daily supplementation with 500–1200 mg of calcium + 700–1000 IU of oral Vitamin D reduce the rate of bone loss in postmenopausal

Bone Formation	The building of new bone through osteoblasts. Bone formation, which is part of the bone remodelling process, includes the synthesis of organic matter (mostly collagen type I) and subsequent mineralisation.
Bone Modelling	The generation and shaping of new bone during the period of skeletal growth.
Bone Remodelling	The continuous and life-long process of renewing bone through the balanced processes of bone resorption and bone formation. Bone remodelling is stimulated by biomechanical and biochemical influences and tightly controlled through circulating osteotropic hormones and local mediators.
Bone Resorption	The removal of mineralised bone by osteoclasts. Bone resorption, which is part of the bone remodelling process, includes the release of mineral (mostly calcium and phosphate) and subsequent proteolysis of organic matter (mostly collagen).

women by 50% and reduce the number of hip and non-vertebral fractures in older and elderly men and women by 30–70%. Data on vertebral anti-fracture efficacy are sparse, but the available evidence indicates moderate efficacy at least in populations with subclinical vitamin D deficiency (= more than 80% of the European/US population aged >70 years). In contrast, “active” vitamin D-metabolites are still controversial in regard to their therapeutic use in postmenopausal and/or age-related (“senile”) osteoporosis.

Strontium salts have long been under investigation as anabolic agents for bone. In animals, strontium stimulates bone formation and substitutes for calcium in hydroxyapatite crystals. In humans, large controlled trials have shown increases in bone mass (after correction of BMD values for strontium content), a significant reduction in vertebral fractures, and a borderline reduction in non-vertebral fractures. The mechanism of action is largely unknown and may involve modulation of the calcium receptor or calcium channels.

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Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMPs) comprise a family of 15 cytokines involved in the growth and differentiation of various tissues and organs such as bone, heart, kidney, eyes, skin and teeth. The members of this family which influence bone remodelling stimulate the differentiation of bone marrow stem cells into bone forming cells. BMPs are currently being tested in clinical trials for their potential to promote union of fractures and healing of bone defects. Among the BMPs being tested, is BMP-7, which also stimulates the

production of erythropoietin (EPO). EPO is produced in the kidney and stimulates the generation of erythrocytes from precursor cells. In the clinic, it is used for the treatment of anemia caused by chronic renal failure.

► Bone Metabolism

Botulinum Toxin

Bacterial Toxins

Botulism

Botulism is a disease caused by ingestion of foods contaminated with *Clostridium botulinum* (food-borne botulism) or, very rarely, by wound infection (wound botulism) or colonization of the intestinal tract with *Clostridium botulinum* (infant botulism). The toxins block the release of acetylcholine. Botulism is characterized by generalized muscular weakness, which first affects eye and throat muscles and later extends to all skeletal muscles. Flaccid paralysis can lead to respiratory failure.

► Bacterial Toxins

► Synaptic Transmission

Bradykinesia

Bradykinesia is slowness and poverty of movement.

► Anti-Parkinson Drugs

Bradykinin

Bradykinin is a nonapeptide enzymatically produced from kallidin in the blood, where it is a potent agent of arteriolar dilation and increased capillary permeability.

Bradykinin is also released from mast cells within damaged tissues. It produces inflammation and activates nociceptors via bradykinin B1 and B2 receptors.

- Kinins
- Analgesics

Brain Derived Neurotrophic Factor

Neurotrophic Factors

Brain Natriuretic Peptide

Natriuretic Peptides

Breast Cancer

Breast cancer is cancer of the breast tissues. The major part of this cancer in women is dependent on the female sex hormones for their growth. An estrogen antagonist can therefore be used in the treatment of breast cancer to limit the growth of the tumor.

- Selective Sex-steroid Receptor Modulators

Bremelanotide

Formerly known as PT-141 from Palatin Technologies is in phase 2 clinical trials. It is a melanocortin receptor agonist and is being trialled for male and female sexual dysfunction and is believed to have none of the cardiovascular effects possibly associated with other therapies.

- Melanocortin

Bronchial Asthma

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Synonyms

Asthma bronchial

Definition

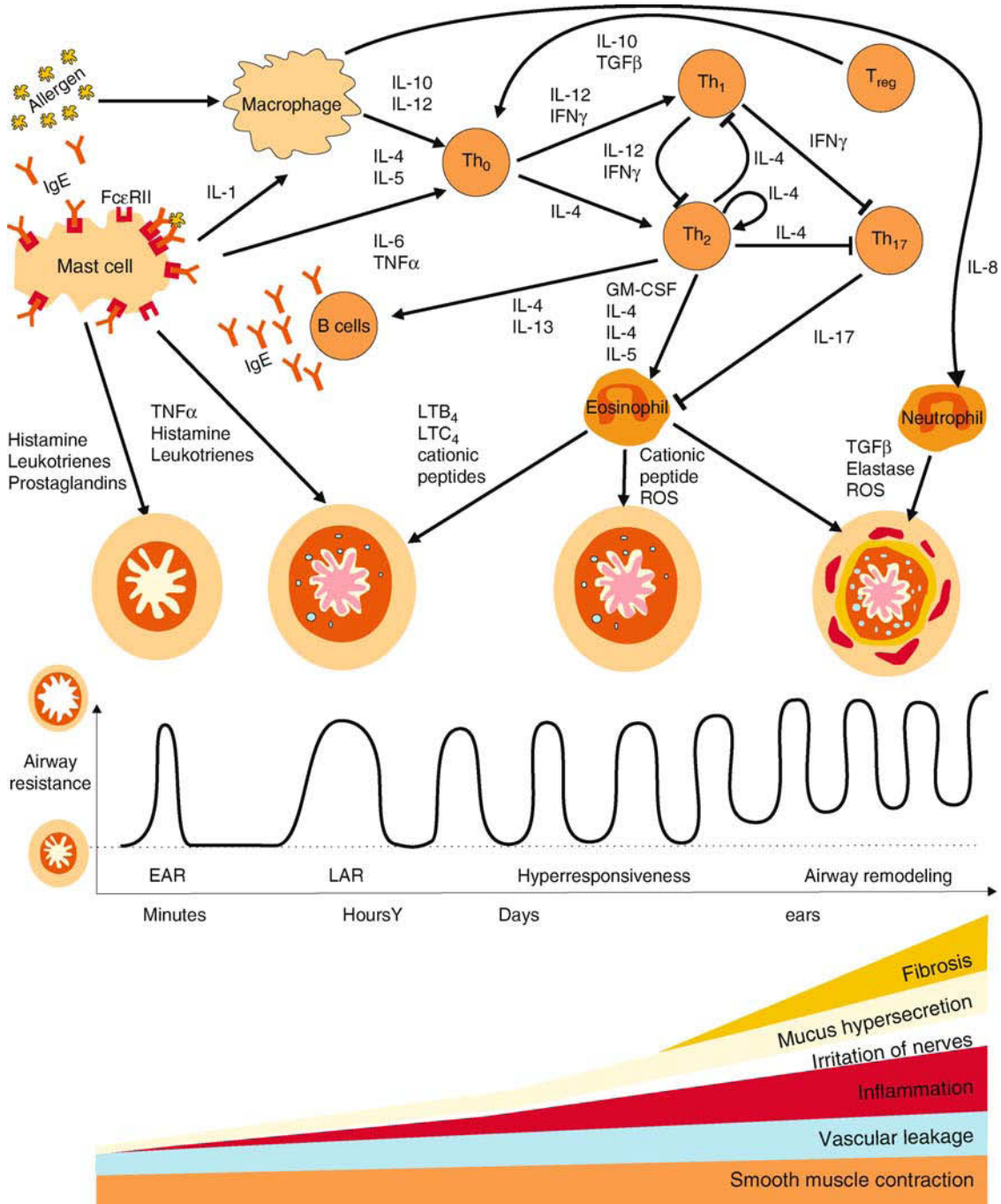
According to the Global Initiative for Asthma (GINA, ►<http://www.ginasthma.org>), asthma affects about 300 million people worldwide. Men of all ages are affected by this chronic airway disorder that, without appropriate treatment, limits quality of life and is sometimes fatal.

Asthma is a chronic inflammatory disorder of the airways associated with recurrent airway obstructions, airway hyperresponsiveness and airway remodeling. Symptoms of asthma include ►wheezing, coughing, chest tightness, and shortness of breathing, particularly at night or in the early morning. Airway obstruction is often reversible either spontaneously or after treatment. Missing or inadequate treatment will result in airway remodeling.

Basic Mechanisms

About 90% of all asthmatics have increased serum IgE levels and suffer from Type I allergy, called extrinsic (allergic) asthma.

Allergic asthma is a chronic inflammatory disorder of the lungs with a prevailing T helper Th2 immune response to ►allergens (Fig. 1). Allergic asthma depends on the presence of IgE bound to high affinity Fcε-receptors (FcεR) on mast cells. Without IgE allergic asthma cannot occur. Thus to understand the primary cause of allergic asthma means to understand why the B-cells of these patients produce IgE specific to the allergen. While this remains unknown at present, the popular hygiene hypothesis holds that allergic asthma is initiated early in life when the immune system is trained. When CD4+ T helper cells encounter pathogens such as viruses or bacteria, these T-helper cells develop into TH1 cells, which produce mainly TNFα and IFNγ. In the absence of such pathogens (which become uncommon with increasing hygiene), CD4+ cells preferentially develop into TH2 cells that produce ►IL-4 and ►IL-5. These so-called TH2 cytokines induce the maturation of naïve T-helper cells into further TH2 cells and of B cells to produce specific IgE antibodies. These responses can be downregulated by IL-10 from regulatory T-cells or macrophages, which modulate TH2 cell maturation and increase tolerance to



Bronchial Asthma. Figure 1 Pathophysiology of asthma. Th2 lymphocytes take a central position in the pathophysiology of asthma. By using various cytokine signals, they inactivate Th1 and Th17 cells, activate eosinophils, and trigger a class switch in B-lymphocytes to make them produce IgE. These IgEs bind to mast cells, where subsequent allergen contact leads to crossbridging and activation of mast cells. The mast cell mediators trigger the early allergic response (EAR), the late allergic response (LAR), the activation of Th2 cells, and other inflammation responses. The inflammatory response mobilizes eosinophils, neutrophils, macrophages, and lymphocytes that infiltrate the lung tissue and sustain the inflammation. Eosinophils release mediators that contribute to the EAR and cytotoxic agents that injure the epithelial layer. Repetitive and chronic inflammation leads to airway remodeling and airway hyperresponsiveness. The curve shows a schematic illustration of airway resistance (as a measure of bronchoconstriction) in the course of asthma showing that the asthma attacks become more frequent and more severe with disease progression. In addition baseline airway resistance increases.

allergens. Recently Th17 cells were discovered to produce IL-17, which downregulates eosinophil chemotaxis. Its release is inhibited by the products of both Th1 and Th2 cells, namely IFN γ and IL-4 [1].

Cardinal Symptoms

The activation of mast cells by allergen initiates the asthma symptoms: within minutes after allergen contact, the early allergic response (EAR), within hours the late allergic response (LAR), and within years and after repeated asthma episodes, chronic airway inflammation, airway remodeling, and airway hyperresponsiveness.

Early and Late Phase in Asthma

Immediate responses to allergen occur within minutes. Allergen, cross-linking IgE bound to mast cells, causes degranulation of preformed (histamine, proteoglycans, serine proteases, TNF) secretion of newly formed mediators (prostaglandins, cysteinyl leukotrienes, thromboxane, $\blacktriangleright$ platelet-activating factor, cytokines). The mixture of histamine, prostaglandins, thromboxane and cysteinyl leukotrienes causes airway smooth muscle contraction.

Frequently, the EAR is followed by a late phase response 4–6 h later and it is caused by the pulmonary sequestration of eosinophils, neutrophils, mast cells, and T-lymphocytes. This leukocyte recruitment depends on mast cell-derived mediators such as TNF α and various chemokines, as well as on the expression of adhesion molecules on leukocytes (e.g. VLA-4, CD11/18) and vascular endothelial cells (e.g. VCAM-1, ICAM-1, E-selectin). Products of these leukocytes have several functions: First, they cause the second phase of bronchoconstriction, mucus secretion, and airway swelling; second, they cause tissue destruction; third, they launch and entertain the chronic inflammation.

Inflammation

Airway inflammation is a characteristic clinical feature of asthma. The distinction between the LAR and chronic inflammation becomes more difficult as the disease progresses. Infiltrated leukocytes release *cytotoxic mediators* such as $\blacktriangleright$ reactive oxygen species (ROS) and $\blacktriangleright$ cationic (basic) proteins causing epithelial damage and *cytokines* that perpetuate the inflammation. Sustained inflammation leads to airway hyperresponsiveness and airway remodeling.

Hyperresponsiveness

Airway hyperresponsiveness is an exaggerated airway narrowing in response to a variety of unspecific stimuli. It can be measured by bronchial provocation with histamine, methacholine or adenosine. The reason for hyperresponsiveness may be stimulation of sensory nerves that are located within the epithelium. They become easily accessible after denudation of the

epithelium. This irritation of nerves leads to reflex smooth muscle contraction, mucus secretion, and edema formation (Fig. 2).

Airway Remodeling

The term “airway remodeling” refers to structural changes in the airways and is the consequence of chronic airway inflammation. The structural changes involve a thickening of the airway wall and this may explain the incomplete reversibility of airway narrowing in asthmatic patients. Hyperplasia of goblet cells coupled with hypertrophy of submucosal glands and increased vascularity of the airway wall amplify mucus secretion and plasma protein leakage, which are responsible for the formation of the characteristic mucus plugs that obstruct the airways. Other characteristic features are smooth muscle hypertrophy and hyperplasia, and subepithelial fibrosis.

Aspirin-Sensitive Asthma

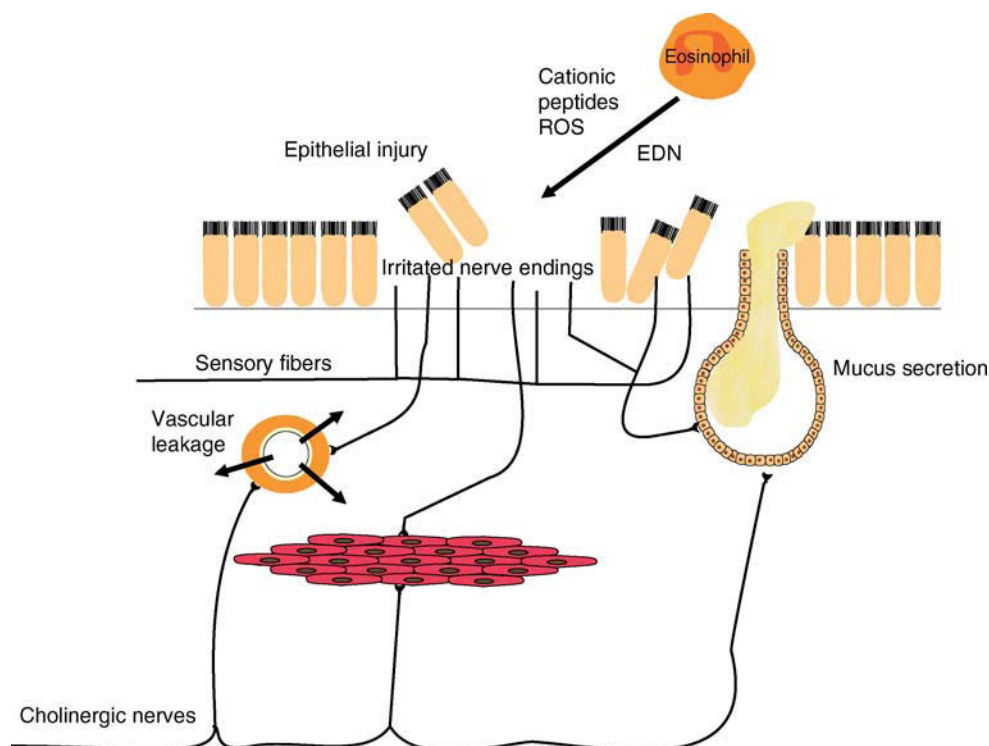
Aspirin sensitive asthma, affecting about 10% of all asthmatics, is a nonallergic response to aspirin and other agents that inhibit cyclooxygenase-1. Mechanistically, the most likely reasons are lack of bronchoprotective prostaglandin E₂ and shunting of arachidonic acid into the leukotriene pathway.

Pharmacological Interventions

Pharmacological therapy depends on the severity of the disease, which is graded into mild, mild persisting, moderate persisting, and severe asthma, depending on the frequency of asthma attacks and the duration of the disease. The pharmacological agents are divided into relievers that lead to a rapid improvement of breathing during asthma attacks (β_2 -agonists, anticholinergics), and controllers (steroids, antileukotrienes, cromones) that control the disease by suppressing the inflammation and exacerbations (Table 1). The inhaled route is preferred. At present, healing of asthma by pharmacological therapy is not possible [2,3,4].

β_2 -Receptor Agonists

β_2 -Receptor agonists relax the bronchial smooth muscle by increasing intracellular cAMP levels, which in turn decrease calcium levels and interfere indirectly with myosin light chain phosphorylation. β_2 -Receptor agonists are very effective bronchodilators and can rescue patients from asthma attacks. β_2 -Receptor agonists are classified according to the duration of their action. Terbutaline, albuterol, and fenterol have a short biological half-life of 2–6 h and are mainly used as the only therapy in nonpersisting asthma and as a standby medication at all other stages of asthma. For other levels of asthma, long-acting bronchodilator such as salmeterol, bambuterol, and aformoterol with half-lives of 15–18 h are used. Typical side effects of this class of drugs are



Bronchial Asthma. Figure 2 Mechanisms of bronchial hyperresponsiveness. Toxic products from eosinophils [cationic peptides, reactive oxygen species (ROS)] cause epithelial injury. Nerve endings become easily accessible to mediators from mast cells, eosinophils [eosinophil-derived neurotoxin (EDN)], and neutrophils, and to airborne toxicants such as SO₂. Activation of nerve endings stimulates effector cells like mucosal glands and airway smooth muscle either directly or by cholinergic reflexes.

interactions with cardiac β_1 -receptors, leading to tremor, arrhythmias, and tachycardia.

Anticholinergics

Cholinergic smooth muscle contraction occurs mainly via M_3 receptors. The clinically used anticholinergics (ipratropium bromide, tiotropium bromide) bind competitively to M_3 and M_2 receptors on airway smooth muscle and mucosal glands, but only tiotropium dissociates quickly from the postsynaptic inhibitory M_2 receptors, contributing to its enhanced inhibitory effect and its prolonged actions (16 h instead of 6 h). However, the lag phase of 30 min after intake limits the usefulness of these drugs in asthma. Inhaled anticholinergics are useful for the treatment of nocturnal asthma and above all for the treatment of COPD. Even when anticholinergics are inhaled, parasympathetic side effects like increased heart rate, soporific effects, and dry mouth may occur.

Methylxanthines

Methylxanthines have relaxing and anti-inflammatory effects. Accumulation of intracellular cAMP by inhibition of PDE3 (phosphodiesterase-3) relaxes airway

smooth muscle, whereas inhibition of phosphodiesterase-4 (PDE4) reduces inflammation. At therapeutic concentrations, methylxanthines inhibit PDE3 activity by only 10–20%. Therefore, other mechanisms have been suggested, e.g., methylxanthines that act as adenosine (A1)-antagonists. Naturally occurring xanthines, like caffeine or theobromine are little effective in the treatment of asthma. At the beginning of the twentieth century, xanthines were widely used as asthma drugs; today theophyllin, bamifyllin, and elixophyllin are still used as an additional therapy for severe asthma (oral) and for status asthmaticus (i.v.). All methylxanthines have a short half-life and a small therapeutic window, which limits their use and necessitates either tight control of plasma levels or the usage of slow release forms.

Phosphodiesterase-4 Inhibitors

In the airways, inhibition of PDE4 is much more anti-inflammatory than bronchodilatory. Although effective in animal experiments, the neuronal and gastric side effects of PDE4-inhibitors have so far impeded their use in humans. Two new orally active PDE4-inhibitors (roflumilast, cilomilast) have shown some effectiveness in advanced clinical trials, but have not yet been approved.

Bronchial Asthma. Table 1 Asthma therapy, according to the German guidelines for asthma and the GINA report 2006 (GINA, ►<http://www.ginasthma.org>). FEV1, forced expiratory volume in 1 s

Phenotype	Therapy on demand	Permanent therapy
Mild nonpersisting asthma		
Occasional asthma attacks, less than 2 per month	Rapid-acting inhaled β_2 -agonist	None
Mild persisting asthma		
Asthma attacks less than twice a week; FEV1 >80%	Rapid-acting inhaled β_2 -agonist	Low dose inhaled glucocorticoid or cromone (children)
Moderate asthma		
Asthma attacks more than twice a week; FEV1 60–80%; daily use of bronchodilators.	Rapid-acting inhaled β_2 -agonist	Inhaled glucocorticoid
		Plus
		Long-acting inhaled β_2 -agonist
		Leukotriene receptor antagonist
		Slow releasing theophyllin
		Anticholinergic
Severe asthma		
Daily asthma attacks, often by night; FEV1 <60% reduced physical stamina; daily use of bronchodilators.	Rapid-acting inhaled β_2 -agonist	Inhaled glucocorticoid
		Plus
		Long-acting inhaled β_2 -agonist
		Plus
		Oral glucocorticoid
		Anti IgE
		Slow releasing theophyllin
<i>Status asthmaticus</i>		
Severe, life-threatening asthma attacks; inhaled β_2 -agonists ineffective	Oxygen	Glucocorticoids oral or i.v.
		Plus
		Theophyllin i.v.
		Plus
		Long-acting β_2 -agonist (oral or subcutaneous)

Anti-Leukotriene Therapy

Leukotrienes have multiple roles in asthma. They cause bronchoconstriction, swelling, and mucus secretion (LTC_4 , LTD_4) as well as eosinophil recruitment (LTE_4). Antileukotriene therapy is based either on inhibition of the 5-lipoxygenase (zileuton; approved only in the USA) or on cys-LT1 receptor antagonism (montelukast, zafirlukast, pranlukast). The anti-inflammatory potential of leukotriene antagonist is smaller than that of steroids. Patients with mild or moderate asthma, especially those who cannot or will not take steroids, are possible candidates for these drugs. Antileukotriene therapy is particularly effective in the treatment of aspirin-sensitive and exercise-induced asthma.

Cromones

Cromones are used to prevent asthma attacks. To be effective they have to be taken continuously. Their mechanism of action is poorly understood. Cromones (cromolyn sodium, nedocromil) act as mast cell-stabilizing agents,

preventing the release and production of histamine and other inflammatory mediators. They do also affect macrophage activation and inflammatory cell recruitment. These compounds diminish hyperresponsiveness in asthma, suppressing the irritant nerve response of denuded pulmonary epithelium. The usage of cromones is declining; they are still used in children (because they have virtually no side effects), and sometimes in adults with moderate asthma.

Anti-IgE

Allergic or extrinsic asthma affects 90% of all asthmatics. The production of IgE by B-cells and the $\text{Fc}\epsilon\text{R}$ -dependent activation of mast cells, eosinophils, basophils, and macrophages is critical for the development of asthma. A clear correlation exists between circulating IgE levels and disease severity. Reduction of circulating IgE antibodies by a humanized mouse anti-IgE antibody (omalizumab) mitigates asthma symptoms. Owing to their long half-life, (bi)monthly subcutaneous antibody

injections are sufficient to repress IgE levels and to reduce asthma symptoms. This (expensive) drug is approved as an add-on therapy to steroids in severe asthma.

Steroids

Asthma is a chronic inflammatory disease. Therefore steroids represent the most important and most frequently used medication. Already after the first treatment, steroids reduce cellular infiltration, inflammation, and the LAR, whereas changes in the EAR require prolonged treatment to lower the existent IgE levels. The mechanisms of steroid actions are complex and only incompletely understood. Besides their general anti-inflammatory properties (see chapter ►[glucocorticoids](#)), the reduction of IL-4 and IL-5 production from T-lymphocytes is particularly important for asthma therapy. The introduction of inhaled steroids, which have dramatically limited side effects of steroids, is considered one of the most important advancements in asthma therapy. Inhaled steroids (beclomethasone, budesonide, fluticasone, triamcinolone, mometasone) are used in mild, moderate, and partially also in severe asthma; oral steroids are used only in severe asthma and the treatment of ►[status asthmaticus](#). Minor side effects of most inhaled steroids are hoarseness and candidiasis, which are avoided by the prodrug steroid ciclesonide.

There is now good evidence that the clinical manifestations of asthma symptoms – impairment of lung function, sleep disturbances, limitations of daily activity, and the use of rescue medications – can be controlled with appropriate treatment. When asthma is controlled, there should be no more than occasional recurrence of symptoms and severe exacerbations should be rare.

- [β-Adrenergic System](#)
- [Muscarinic Receptors](#)
- [Phosphodiesterases](#)
- [Steroids](#)
- [Allergy](#)

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Btk

Btk (Bruton's tyrosine kinase) is a phosphatidylinositol 3'-kinase sensitive cytoplasmic tyrosine kinase. Germ-line loss of function mutations of Btk cause X-linked agammaglobulinaemia in human and X-linked immunodeficiency in mice.

- [Phospholipid Kinases](#)
- [Tyrosine Kinases](#)

Butyrophenones

Antipsychotic Drugs

Butyrylcholinesterase

- [Cholinesterases](#)

C₂ Domain

C₂ domains (phosphokinase C conserved 2 domains) mediate membrane targeting of diverse peripheral proteins. A C₂ domain consists of approximately 130 residues and was first discovered as the Ca²⁺-binding site in conventional phosphokinase Cs.

- ▶ Phospholipid Kinases
- ▶ Ca²⁺-binding Proteins
- ▶ Adaptor Proteins

C Kinase

- ▶ Protein Kinase C

C-reactive Protein

An acute-phase reactant protein, the plasma concentration of which increases in inflammatory states.

- ▶ Atherosclerosis

Ca²⁺-ATPase

The Ca²⁺-ATPase transports Ca²⁺ ions into endoplasmic reticulum or out of the cell from the cytoplasm, using the energy of ATP hydrolysis.

Ca²⁺-binding Proteins

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Definition

These proteins can be subdivided into extracellular and intracellular Ca²⁺-binding proteins (Table 1).

Members of the Extracellular Proteins

▶ **Noncollagenous Ca²⁺-binding proteins** bind to bone minerals. They contain stretches of γ -glutamic acid residues necessary for Ca²⁺-binding.

▶ **EGF-like domains** were identified in extracellular proteins such as fibrillin-1. EGF-modules contain about 40–45 amino acids including six cysteine residues which normally build S–S disulfide bond bridges. Mutations in the fibrillin-1 gene cause Marfan syndrome and related disorders.

▶ **Ca²⁺ sensing receptor**, a member of G-protein coupled receptors, is composed of seven transmembrane spanning domains. The extracellular domain contains clusters of negatively charged amino acids sensing even small fluctuations of extracellular calcium. Mutations in this receptor cause inheritable hypo- and hypercalcemic syndromes.

Members of the Intracellular Ca²⁺-Binding Proteins

Non-EF-hand Ca²⁺-binding proteins including the annexins, C2-domain proteins, and gelsolin, and the EF-hand Ca²⁺-binding proteins.

This review will focus on the large family of EF-hand proteins characterized by a common structural motif, the EF-hand (Fig 1).

Basic Characteristics of the EF-Hand Ca²⁺-Binding Proteins

Structures and Functions

These calcium-binding proteins form a superfamily of proteins containing more than 600 members [1–3]. The

Ca^{2+} -binding Proteins. Table 1 Families of Ca^{2+} -binding proteins

Extracellular Ca^{2+} -binding proteins (a)
– Noncollagenous proteins (osteocalcin, osteopontin, osteonectin)
– EGF-like domain proteins (fibrillin)
– Ca^{2+} -sensing receptor
Intracellular Ca^{2+} -binding proteins
– EF-hand Ca^{2+} -binding proteins (b)
– Ca^{2+} -buffer proteins
– Ca^{2+} -regulatory proteins
– Penta EF-hand proteins
– Calcineurins
Non-EF-hand Ca^{2+} -binding proteins (c)
– Annexins
– C2 domain proteins
– Gelsolin

References: (a) Hofer et al (2003), *Nat Rev Mol Cell Biol* 4:530–538; Handford et al (2000), *Biochim Biophys Acta* 1498:84–90. (b) Kawasaki et al (1998), *Biomaterials* 11:277–295; Ikura and Ames (2006) *Acad Sci USA* 103:1159–1164; Krebs and Heizmann (2007), *New Comprehensive Biochemistry* (Series, Bernardi G) Elsevier, pp 51–93; Heizmann and Hunziker (1991), *TIBS* 16:98–103; Lambers et al (2006), *EMBO J* 25:2978–2988; Dagher et al (2006), *Biochim Biophys Acta* 1763:1250–1255; Aramburu et al (2000), *Curr Top Cell Regul* 36:237–295; Mikhaylova et al (2006), *Biochim Biophys Acta* 1763:1229–1237; Zatz and Starling (2005), *N Engl J Med* 352:2413–2423. (c) Gerke et al (2005), *Nat Rev Mol Cell Biol* 6:449–461; Shin et al (2005) *J Biol Chem* 280:1641–1651; Choe et al (2002), *J Mol Biol* 324:691–702; Maki et al (2002), *Biochim Biophys Acta* 1600:51–60.

human genome alone may contain up to 100 EF-hand containing proteins. They are characterized by the EF-hand, or helix-loop-helix motif (Fig 1). The members of this family can be subdivided into several categories (Table 1). The first group of proteins are the calcium-buffering proteins including parvalbumin, calbindin $\text{D}_{28\text{K}}$, and calretinin. The second group is composed of the calcium-regulatory sensor proteins that are involved in the transmission of the calcium signal. This group consists of calmodulin, the S100 proteins, and the Ca^{2+} -sensor proteins. The other two groups are the penta EF-hand proteins and the calcineurins (CaNs).

Calcium-Buffering Proteins

Parvalbumin

►Parvalbumin (Fig 1) is a cytosolic protein expressed in fast-twitch skeletal muscles and in the nervous system. In muscles, parvalbumin controls the relaxation process. In the CNS, parvalbumin, expressed in a subpopulation of GABAergic neurons, is correlated with their firing rates, protecting the cells from Ca^{2+} overload.

Calbindins and Calretinin

Two types of ►calbindins have been described, calbindin $\text{D}_{28\text{K}}$ and calbindin $\text{D}_{9\text{K}}$. The latter (now renamed as S100G) belongs to the S100 protein family.

Both proteins, hormonally controlled by vitamin D, are expressed in the kidney, intestine, and brain. Calbindin $\text{D}_{28\text{K}}$ contains six EF-hands, four of which have a high affinity for Ca^{2+} . Calbindin $\text{D}_{28\text{K}}$ facilitates Ca^{2+} diffusion in Ca^{2+} -transporting epithelial cells and provides protection against toxic Ca^{2+} levels by buffering the cytosolic Ca^{2+} concentrations during high Ca^{2+} influx. Recent studies have indicated that this protein may also act as a Ca^{2+} sensor (Table 1).

In the CNS, calbindin $\text{D}_{28\text{K}}$ is widely expressed in a subpopulation of neurons distinct from those immunoreactive for parvalbumin or calretinin.

Calretinin is homologous to calbindin $\text{D}_{28\text{K}}$. It is abundantly expressed in the central and peripheral nervous system and other organs. The protein contains four EF-hand domains homologous to the first four of calbindin $\text{D}_{28\text{K}}$.

Ca^{2+} Regulatory Proteins

Troponin (subunits TN-T, TN-I, TN-C)

The Ca^{2+} -binding subunit TN-C is homologous to calmodulin with four EF-hands. In contrast to calmodulin, which is ubiquitously expressed in multicellular eukaryotic organisms and interacts with many targets, ►troponin specifically regulates muscle contraction. There are some structural differences between Troponin C in skeletal and cardiac muscles reflecting their physiological differences.

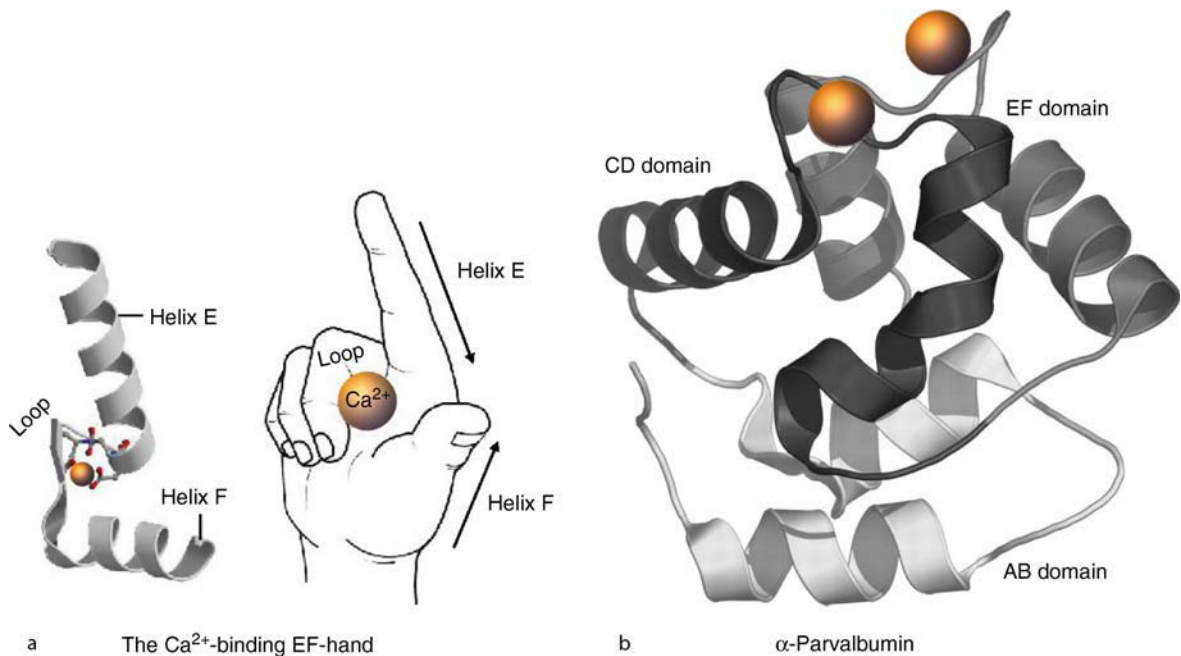
Calmodulin (CaM)

CaM is ranked among the most conserved proteins known to date, which also include proteins like histones or actin. CaM possesses four EF-hand calcium-binding sites distributed in pairs separated by a long central helix. CaM can regulate its target proteins in a calcium-dependent or -independent manner. These targets have been classified into “classic” and “nonclassic” (proteins with the IQ motif). The list of the “nonclassic” targets of CaM has grown exponentially in the last few years because of the screening of the genome sequence database.

CaM plays a key role in many cellular processes. In the CNS, it is involved in synaptic transmission and neuronal plasticity associated with short-term and long-term potentiation, and learning and memory processes.

Centrins

►Centrins are calmodulin-like proteins that have an important function in the organization and duplication of microtubules. Like CaM, centrin is also comprised of two structurally independent globular domains connected by a flexible tether, and each domain is



Ca²⁺-binding Proteins. Figure 1 (a) The EF-hand consists of helix E, followed by a loop (coordinating the Ca²⁺-ions) and a second helix F. This structure can be mimicked by thumb, forefinger, and bent middle finger (Ca²⁺-loop). (b) The first X-ray structure of carp parvalbumin (PV) was reported by Kretsinger and Nockolds (1973), *J Biol Chem* 248:3313. α-PV consists of six α-helices (labeled A-F) connected by loops. The helices associate in pairs to form three domains labeled AB, CD, and EF. Ca²⁺-ions are bound in the helix-loop-helix, or EF-hand, substructures of the CD-EF domain (ribbon drawing modified from Bottoms et al (2004), *Protein Sci* 13:1724–1734). For further structures, please prefer to the RCSB Protein Data Bank (PDB): ► <http://www.pdb.org/>

composed of two EF-hand type calcium binding domains. However, in contrast to CaM, most centrin contain one or more nonfunctional calcium-binding sites.

Neuronal Sensor Proteins (NCS)

This family contains more than 40 members subdivided into five subfamilies [4]. The NCS have been involved in phototransduction and regulation of neurotransmitter release. The NCS have two pairs of EF-hands and, unlike CaM and S100 proteins, possess a consensus myristoylation sequence at the N-terminal responsible for the targeting of the NCS to the membrane.

Members of the first group of NCS are ► **recoverin** and ► **visinin**. Recoverin is a 23 kDa myristoylated protein found under normal conditions only in photoreceptor cells (rods and cones). The main function of recoverin is to bind to and inhibit rhodopsin kinase, thereby prolonging the light response.

A second class of neuronal calcium sensors is formed by the guanylate cyclase-activating protein (GCAP). The GCAPs are expressed only in the photoreceptor cells of the retina of vertebrates. Recoverins and GCAPs have antagonistic roles in phototransduction.

Two other NCS families, i.e., frequenins and KChIPs, are thought to regulate the release of

neurotransmitters, the biosynthesis of polyphosphoinositides, and the activity of Kv channels (KChIPs), respectively.

VILIP-1, VILIP-2, hippocalcin, neurocalcin, and VILIP-3 (also named hippocalcin-like 1 protein) are expressed in different cell types in the brain. All isoforms are neuronal-specific.

Recently, two novel sensor proteins, caldendrin and calneuron have been described. Caldendrin is abundantly expressed in neurons and is thought to play an important role in different aspects of synapto-dendritic Ca²⁺ signaling. Calneurons are homologues to caldendrin, have a different EF-hand organization, compared to other calcium sensor proteins and are prominently expressed in neurons. Despite some significant structural differences they are possibly involved in similar Ca²⁺-regulated processes as caldendrin and other neuronal calcium sensor proteins.

The Penta EF-Hand (PEF) Family

These cytosolic proteins contain five EF-hand domains and are able to translocate to the plasma membrane upon calcium binding [5]. In addition to the EF-hand domains, these proteins also have a hydrophobic glycine/proline-rich domain, important for their translocation to the membrane. To date five members of this

family have been described in human tissues, such as sorcin, calpain light and heavy chain, grancalcin, apoptosis-linked gene-2 (ALG-2), and peflin.

Sorcin (soluble resistance-related calcium binding protein) was isolated from multidrug-resistant cells and is expressed in a few mammalian tissues such as skeletal muscle, heart, and brain. In the heart, sorcin interacts with the ryanodine receptor and L-type Ca^{2+} -channels regulating excitation in contraction coupling.

► **Calpains** belong to the family of cytosolic calcium-dependent cysteine proteinases and are involved in cell cycle regulation and apoptosis, and in pathophysiological processes such as Alzheimer's and Parkinson's disease. At least 14 members of the calpain family have been identified, which can be either tissue-specific or ubiquitously expressed. Most calpains are composed of heterodimers with a large catalytic subunit and a small regulatory subunit. Both large and regulatory subunits contain the penta EF-hand motif.

Calpain-10 (CAPN10) is the first diabetes gene to have been identified through a genome scan. The discovery of calpain-10 has identified it as a molecule of importance to insulin signaling and secretion that may have relevance to the future development of novel therapeutic targets for the treatment of type 2 diabetes.

ALG-2 is the first calcium-binding protein of the EF-hand family found to be directly involved in apoptosis. ALG-2 is a 22 kDa protein and like the other members of the penta EF-hand family, contains five EF-hands, with only two of them functional. ALG-2 protein is expressed in the brain and eye and was found to be upregulated in various cancer tissues. Several targets have been found, such as proteins AIP, Alix, peflin, and annexins, suggesting a putative role of ALG-2 in apoptosis.

Peflin presents 40.9% identity with ALG-2. The protein has a long hydrophobic N-terminal extension containing nine nonapeptide repeats. Peflin is ubiquitously expressed. The function of peflin is still unknown, although recent experiments have shown a role in apoptosis.

Calcineurin

► **Calcineurin** is not related to any of the family of the EF-hand calcium-binding proteins mentioned above. It is a protein phosphatase (2B) expressed in many tissues, but selectively enriched in neurons. CaN consists of a catalytic subunit (CaNA) and a regulatory subunit (CaNB). Activation of CaN requires both the interaction of Ca^{2+} /calmodulin with the catalytic subunit and the binding of calcium to the regulatory subunit CaNB. CaNB possesses four functional EF-hands which bind calcium with micromolar affinity. In the brain, CaN regulates a large variety of proteins such as transcription factors, calcium channels, GABA, and glutamate receptor.

CaN Homologous Protein

Calcineurin homologous protein (CHP) is ubiquitously expressed and has four EF-hand domains and one putative site of myristoylation.

Calmyrin is closely related to human calcineurin B. Calmyrin interacts with presenilin 2 and may thus be involved in Alzheimer's disease.

Drugs

Disease Association and Molecular Drug Targets

Proteins of this family are closely associated with human diseases. They have also been proven to be valuable in diagnosis and are considered to have potential as drug targets to improve therapies.

► **Parvalbumin** was found to be a major fish allergen in approximately 70% of fish allergies, mediated by parvalbumin IgE antibodies, leading to respiratory and gastrointestinal symptoms. Parvalbumin also became of interest because of its potential use in gene therapy to correct cardiomyopathy.

► **Troponin** is a regulator of striated muscle contraction. Measurements of troponin I levels are routinely used in the diagnosis of myocardial infarction. In addition, mutations in the troponin I subunit are associated with familial hypertrophic cardiomyopathy.

► **Calmodulin** is involved in several human diseases including Parkinson's disease. Recently, new calmodulin ligands have been developed, which may become a key target for pharmacological interactions.

► **Recoverin** is aberrantly expressed in malignant tumors localized outside the nervous system. This was found to trigger the immune system resulting in the production of autoantibodies inducing the degeneration of the retina.

► **Sorcin** is associated with the development of multidrug resistances in leukemia and other cancers. Sorcin is also able to improve cardiac contractility independently of β -adrenergic stimulation and may prove beneficial in treatment of heart failure. A point mutation in sorcin causes familial hypertrophic cardiomyopathy.

► **Calpain**: Tissue specific calpains have been implicated in diabetes, cataracts, multiple sclerosis, and limb-girdle muscular dystrophy type 2A. More than 50 inhibitors of calpain have been described which have a potential for therapeutic applications.

► **Calcineurin** is involved in cardiac hypertrophy and in cognitive and behavioral defects in the brain. Inhibitors of calcineurin such as cyclosporine A and FK 506 are used clinically in transplant rejection and autoimmune diseases.

Conclusion

Ca^{2+} and Ca^{2+} -binding proteins regulate a plethora of vital physiological functions and many diseases are

linked to abnormalities in the intracellular calcium sensing pathways regulated by Ca²⁺-binding proteins. These proteins have a great potential as drug targets to improve therapies in the future.

- Ca²⁺-sensing Receptor
- Calpains
- Protein Phosphates
- S100 Proteins

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Ca²⁺ Channel

An oligomeric protein that spans a cell membrane forming a regulated pore through which Ca²⁺ can pass. Ca²⁺ channels differ considerably in their selectivity for Ca²⁺ over other cations: For example IP₃R are poorly selective, voltage-dependent Ca²⁺ channels are very selective.

- IP₃ Receptors
- Voltage-dependent Ca²⁺ channels

Ca²⁺ Channel Antagonists

- Ca²⁺ Channel Blockers

Ca²⁺ Channel Blockers

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Synonyms

Ca²⁺ channel antagonists

Definition

Ca²⁺ is an important intracellular second messenger that controls cellular functions including muscle contraction in smooth and cardiac muscle. Ca²⁺ channel blockers inhibit depolarization-induced Ca²⁺ entry into muscle cells in the cardiovascular system causing a decrease in blood pressure, decreased cardiac contractility, and antiarrhythmic effects. Therefore, these drugs are used clinically to treat hypertension, myocardial ischemia, and cardiac arrhythmias.

Mechanism of Action

Voltage-gated Ca²⁺ channels are Ca²⁺-selective pores in the plasma membrane of electrically excitable cells, such as neurons, muscle cells, (neuro) endocrine cells, and sensory cells. They open in response to membrane depolarization (e.g., an action potential) and permit the influx of Ca²⁺ along its electrochemical gradient into the cytoplasm.

Ca²⁺ Channel Physiology

The resulting increase in intracellular free Ca²⁺ triggers and/or modulates important physiological processes. Ca²⁺ influx into nerve terminals, sensory or endocrine cells initiates neurotransmitter or hormone release, respectively (stimulus-secretion coupling). Ca²⁺ entry into the soma and proximal dendrites of nerve cells leads to the activation of intracellular pathways affecting gene transcription (excitation transcription coupling) and neuronal plasticity. In smooth muscle and cardiac myocytes Ca²⁺ influx through these channels induces muscle contraction (excitation contraction coupling).

Ca²⁺ Channel Function

Like other ►voltage-gated cation channels, Ca²⁺ channels exist in at least three states: A resting state stabilized at negative potentials (such as the resting potentials of most electrically excitable cells) that is a closed state from which the channel can open. The open state is induced by depolarization. Channels do not stay open indefinitely because they are “turned off” during prolonged depolarization by transition into an inactivated state. Inactivation is driven both by depolarization

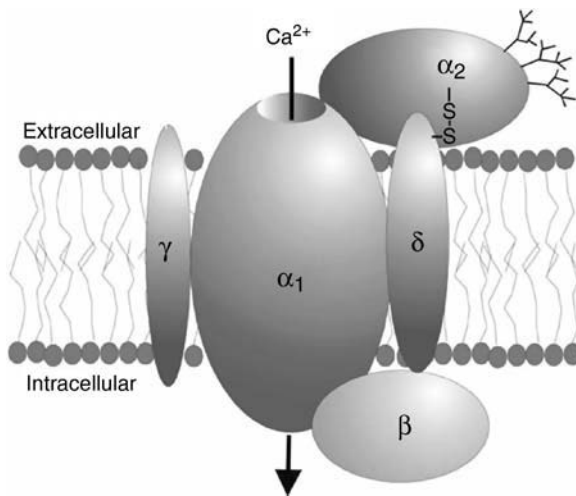
as well as the permeating Ca^{2+} ions (Ca^{2+} -dependent inactivation), thus preventing cellular Ca^{2+} overload during prolonged stimulation. Once the cell repolarizes, inactivated channels return to the resting state and are now again available for opening. Ca^{2+} channel blockers can inhibit Ca^{2+} flux not only by obstructing the open pore but also by “allosterically” stabilizing inactivated closed states. By delaying its transition to the resting state after repolarization drugs can also increase the “refractory period” of these channels.

Ca^{2+} Channel Types

In order to accomplish these diverse physiological tasks described above, nature has created at least five different types of Ca^{2+} channels. These are termed L-, N-, P/Q-, R-, and T-type. Although they are all structurally similar (Fig. 1) they differ with respect to their biophysical properties. Some of them need only weak depolarizations to open and inactivate fast (e.g., T-type Ca^{2+} channels), whereas others require strong depolarizations and inactivate more slowly (e.g. P- or L-type Ca^{2+} channels). Channel types also differ with respect to their sensitivity to drugs. This selectivity is exploited for pharmacotherapy.

L-Type Ca^{2+} Channel Blockers

At present, only organic blockers of L-type Ca^{2+} channels (also termed “ Ca^{2+} antagonist”) are licensed



Ca^{2+} Channel Blockers. Figure 1 Most voltage-gated Ca^{2+} channels exist as a hetero-oligomeric complex of several subunits. α_1 subunits form the Ca^{2+} -selective ion pore and contain the voltage-sensors of the channel. In the case of L-type Ca^{2+} channels, they also carry binding sites for Ca^{2+} antagonist drugs. The accessory α_2 - δ , β , and γ subunits stabilize Ca^{2+} channel function and support its targeting to the plasma membrane. Notably other proteins can associate with the channel complex allowing the formation of signaling complex important for channel targeting and modulation.

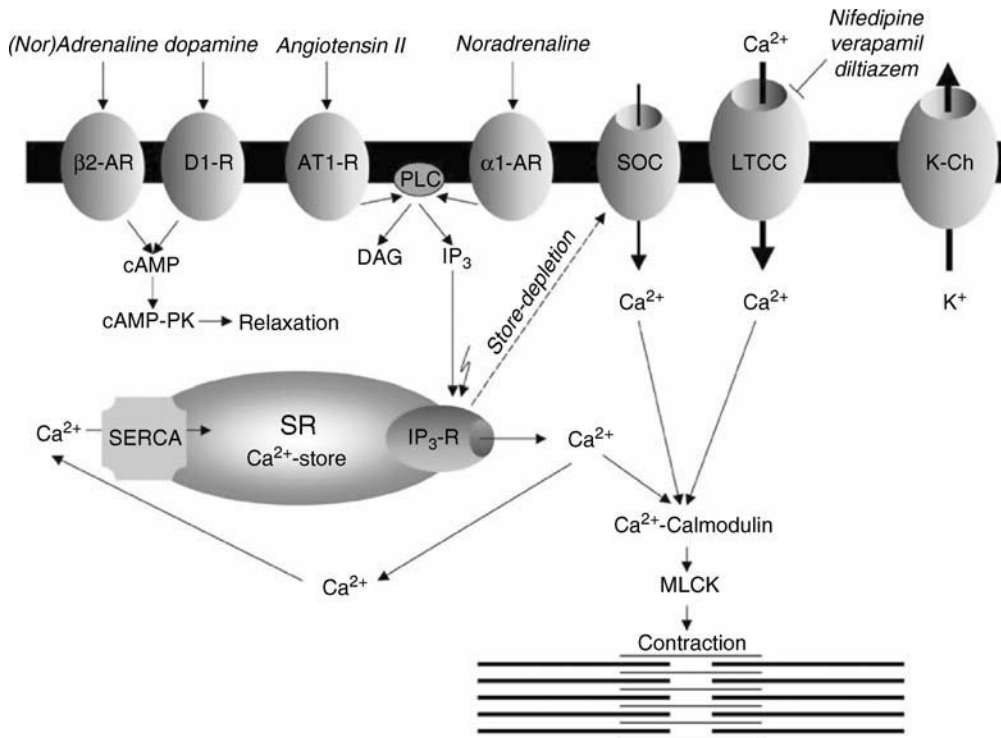
for clinical use. They belong to the most frequently prescribed drugs worldwide. L-type Ca^{2+} channels are the major channel type in muscle cells mediating contraction. Although they do not support fast neurotransmitter release from nerve terminals during short action potentials L-type channels provide Ca^{2+} for neurotransmitter release sites in sensory (cochlear hair cells, retinal photoreceptors) and endocrine cells (insulin secretion in **pancreatic β cells**) and contribute to brain function by coupling synaptic activity to gene-transcription in neurons. Despite these multiple functions, in vivo L-type Ca^{2+} channel block by therapeutic concentrations only causes pharmacological effects in the cardiovascular system.

Signaling pathways controlling cardiac and smooth muscle contraction are depicted in Fig. 2 and Fig. 3. By blocking L-type channels in arterial smooth muscle they reduce Ca^{2+} influx during depolarization. Thus less Ca^{2+} is available for activation of calmodulin, which activates myosin-light chain kinase and thereby turns on actin–myosin interaction (Fig. 2). Note that smooth muscle also contracts after stimulation of receptor-activated pathways. Agonists of angiotensin AT1 (e.g., angiotensin II) and α_1 -adrenergic receptors (e.g., noradrenaline) release Ca^{2+} from intracellular IP_3 -sensitive stores (Fig. 2). Noradrenaline-induced contractions are much less sensitive to Ca^{2+} channel blockers. The differential contribution of depolarization-induced and receptor-activated contraction in different types of smooth muscle and under different pathophysiological conditions is one of the explanations why Ca^{2+} channel blockers are not effective muscle relaxants in other diseases (such as bronchial asthma or urethral spasms). In the heart, Ca^{2+} entering through L-type channels during the action potential serves as a trigger (“trigger Ca^{2+} ”) for further Ca^{2+} release from the sarcoplasmic reticulum that initiates contraction (Fig. 3). β -adrenergic receptor activation increases inotropy at least in part by cAMP-dependent phosphorylation of L-type channels thereby increasing Ca^{2+} entry.

Three different chemical classes of organic Ca^{2+} channel blockers can be distinguished: Dihydropyridines (DHPs; prototype nifedipine), phenylalkylamines (prototype verapamil), and benzothiazepines (prototype diltiazem). Despite their different structure they all bind within a single drug binding domain close to the pore of the channel. In radioligand binding experiments these drugs reversibly interact with this domain in a stereoselective manner and with **dissociation constants** in the (sub)nanomolar range (0.1–50 nM).

DHPs

Widely used DHPs are nifedipine, amlodipine, nitrendipine, nisoldipine, nicardipine, and isradipine. They directly bind to and stabilize the inactivated state of the

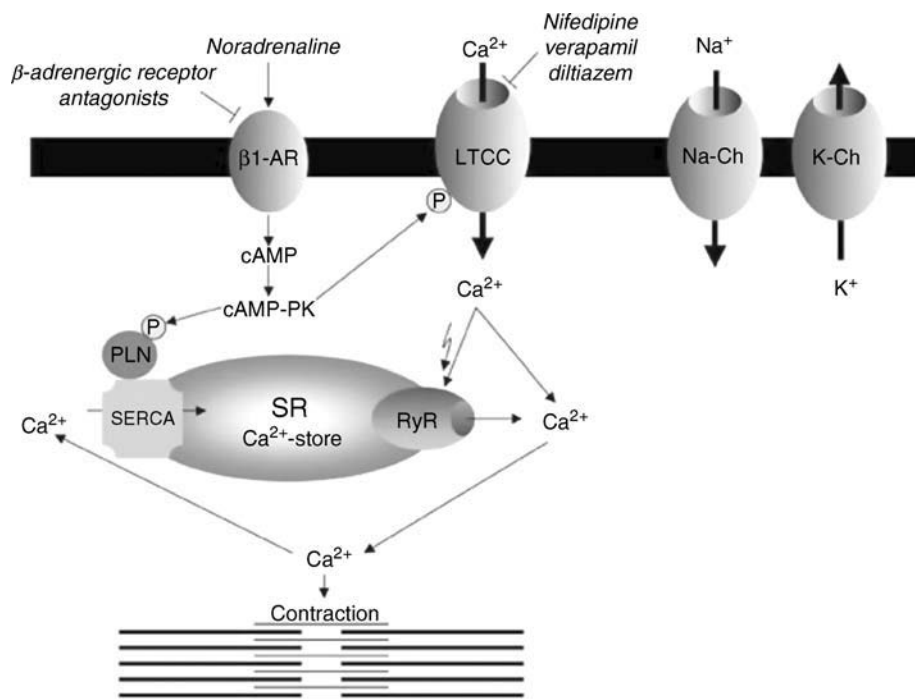


Ca²⁺ Channel Blockers. Figure 2 Simplified view of the pharmacological action of L-type Ca²⁺ channel blockers in arterial smooth muscle: In contrast to cardiomyocytes, action potentials are not carried by fast sodium channels in smooth muscle and depolarizations are more long lasting. Contraction requires the binding of Ca²⁺ to calmodulin, which then activates myosin light chain kinase (MLCK). MLCK phosphorylates the light chain of myosin, which initiates contraction. The Ca²⁺ for activation of this pathway can enter through L-type Ca²⁺ channels in response to depolarization. Ca²⁺ channel blockers inhibit this pathway through concentration-dependent block of Ca²⁺ entry. Alternatively, Ca²⁺ can be released from intracellular stores after activation of membrane receptors (e.g., of angiotensin II AT1 or α_1 -adrenergic receptors) coupled to IP₃ production. IP₃ opens IP₃ receptor channels, RyR related Ca²⁺ release channels in the SR. This process does not involve L-type Ca²⁺ channels and is not inhibited by Ca²⁺ channel blockers. Store-depletion also triggers the activation of “store-operated channels” (SOC) in the plasma membrane, which are also not sensitive to Ca²⁺ channel blockers. SOC can depolarize the plasma membrane and may thus link receptor-activation to the modulation of Ca²⁺ channel activity. Receptor-mediated activation of cAMP-dependent protein kinase (cAMP-PK) results in muscle relaxation through different mechanisms. D1-R, dopamine1 receptor; AR, adrenergic receptor; PLC, phospholipase C.

channel and do not require the channel to open in order to access the binding domain. Inactivated channels are more likely to exist in arterial vascular smooth muscle because depolarizations are longer lasting than in cardiac muscle. Moreover, the arterial smooth muscle channel differs slightly from the cardiac isoform (► [alternative splicing](#) of α_1 subunits) which facilitates channel block by DHPs. As a consequence, DHPs block the channels in arterial smooth muscle at lower concentrations than cardiac muscle. Their clinical use is therefore related to their vasodilating properties in arterial smooth muscle (including the coronary arteries) and not to direct actions on the myocardium and the conduction system (i.e., antiarrhythmic and cardiodepressive effects), which are observed at higher concentrations in vitro or at toxic plasma levels.

Phenylalkylamines

Verapamil is the most widely used phenylalkylamine. Methoxyverapamil (gallopamil) is slightly more potent and licensed for clinical use in some countries. Verapamil mainly gets access to the binding domain when the channel is open. As an organic cation it blocks the channel by interfering with Ca²⁺ ion binding to the extracellular mouth of the pore. Once bound to the open state it can promote the inactivated channel conformation. Verapamil also slows the recovery of channels from inactivation. This increases the refractory period of the drug-bound channel. As a consequence, the number of channels available for Ca²⁺ influx decreases when the time between depolarizations shortens (i.e., when stimulation frequency increases). The open channel block and slowing of recovery explains why inhibition by a



Ca^{2+} Channel Blockers. Figure 3 Simplified view of the pharmacological action of L-type Ca^{2+} channel blockers in cardiac myocytes: In cardiac myocytes, L-type Ca^{2+} channels open when the plasma membrane is depolarized by an action potential carried along the muscle cells by the opening of voltage-gated sodium-channels (Na-Ch). The action potential is terminated (an its duration is determined) by the opening of potassium channels (K-Ch). Ca^{2+} influx triggers massive release of Ca^{2+} from intracellular stores by opening ryanodine-sensitive Ca^{2+} channels (ryanodine receptors, RyR) in the sarcoplasmic reticulum, resulting in an intracellular Ca^{2+} transient. Ca^{2+} influx and released Ca^{2+} directly initiate contraction. Contraction is terminated by the rapid uptake into the SR by SR Ca^{2+} ATPases (SERCA). β -adrenergic receptor stimulation increases inotropy by phosphorylation (P) of phospholamban (PLN) and L-type channels through cAMP-dependent protein kinase (cAMP-PK). The resulting stimulation of Ca^{2+} influx and Ca^{2+} -pump activity increases the load of Ca^{2+} in the SR stores. This leads to enhanced Ca^{2+} transients upon depolarization. Inhibition of Ca^{2+} influx through L-type Ca^{2+} channels by Ca^{2+} channel blockers causes decreased Ca^{2+} entry and SR load. Less Ca^{2+} influx and release results in smaller Ca^{2+} transients and a decrease in contractile force.

given verapamil concentration increases at higher heart rates. Like the lidocaine block of voltage-gate sodium channels, the verapamil block of Ca^{2+} channels becomes more pronounced during tachyarrhythmias. These antiarrhythmic effects of phenylalkylamines are exploited in addition to its vasodilating and cardio-depressive actions.

Benzothiazepines

Diltiazem is the only benzothiazepine in clinical use. Its molecular mechanism of action as well as its pharmacological effects closely resemble those of phenylalkylamines.

All three classes also inhibit depolarization-induced contraction of venous smooth muscle in vitro. However, venous relaxation does not substantially contribute to the hemodynamic actions of Ca^{2+} channel blockers.

Clinical Use (Including Side Effects)

DHPs are potent arterial vasodilators. They act on resistance vessels and therefore reduce peripheral vascular resistance, lower arterial blood pressure, and antagonize vasospasms in coronary or peripheral arteries. By reducing afterload, DHPs also reduce cardiac oxygen demand. Together with their vascular spasmolytic effect, this explains most of the beneficial actions of DHPs in angina pectoris. Most DHPs are only licensed for the therapy of hypertension, some of them also for the treatment of angina pectoris and vasospastic (Prinzmetal) angina.

Fast DHP-induced lowering of blood pressure results in compensatory sympathetic activation and a subsequent increase in heart rate and cardiac oxygen demand. This unfavorable effect has been mainly associated with the use of short-acting DHPs, such as nonretarded formulations of nifedipine, nitrendipine, or

nicardipine. The use of such formulations that cause fluctuations in plasma levels upon multiple dosing is discouraged. Instead, DHPs with slower onset and longer duration of action (amlodipine or slow release formulations of e.g. nifedipine, nisoldipine) are recommended. Due to their vasodilating properties in the absence of negative inotropic actions, DHPs have also been evaluated as vasodilators for the treatment of congestive heart failure in addition to standard therapy. Although long-acting DHPs seem to be safe in these patients, they are not recommended for this indication.

In addition to these vasodilatory and antispastic properties, therapeutic doses of verapamil and diltiazem also exert negative inotropic, dromotropic, and chronotropic actions. As a consequence, compensatory tachycardia does not occur and heart rate may even decrease. Similar to β -adrenergic antagonists, verapamil and diltiazem inhibit exercise-induced increases in heart rate and myocardial oxygen consumption. Therefore both drugs are licensed for the treatment of angina, vasospastic angina, and hypertension. Their negative dromotropic and antiarrhythmic properties (see above) can be exploited to slow AV-conduction and to treat supraventricular arrhythmias. In patients with normal contractile function, the negative inotropic action of verapamil is partially compensated by the decreased afterload and improved myocardial perfusion. However, verapamil may decrease left ventricular function in patients with congestive heart failure. Unlike β -adrenergic blockers, verapamil and diltiazem are not recommended in cardiac failure and for early treatment or secondary prevention of myocardial infarction.

DHPs are also used to treat vasospasms of peripheral arteries (e.g., ►Raynaud's phenomenon) and ►pulmonary hypertension.

Side Effects

Many unwanted effects are related to the vasodilatory effects of Ca²⁺ channel blockers, such as flushing, headache, dizziness, and hypotension. DHPs frequently cause edema and ankle swelling upon chronic use. Constipation is a frequent side effect of verapamil due to its inhibitory action on intestinal smooth muscle. Bradycardia, atrioventricular block, or a decrease in left ventricular function are observed with verapamil (and to a lesser degree diltiazem), especially in patients taking β -adrenergic blockers or who have preexisting cardiac disease (impaired left ventricular function, atrioventricular block). Worsening of angina has also been observed with DHPs. This is most likely due to their effect on coronary resistance vessels resulting in coronary steal in the presence of hypoperfused regions. It may also be caused by the reactive sympathetic activation with increase in heart rate and cardiac oxygen consumption.

Epidemiological and case-control studies suggested that Ca²⁺ channel blockers cause increased risk for

myocardial infarction, cancer, and gastrointestinal bleeding. The increased cardiovascular morbidity was again associated with short-acting DHPs and fast release forms of verapamil and diltiazem. It was explained by the unfavorable hemodynamic effects of short-acting drugs. Enhanced cardiovascular morbidity has not been consistently shown for long-acting formulations. The increased risk of cancer and gastrointestinal bleeding was not confirmed in other large trials.

Ca²⁺ channel blockers cause no side effects expected from channel block in other tissues (e.g., cochlear inner hair cells, retinal photoreceptors or neurons, pancreatic β -cells). Despite the dependence of insulin secretion on L-type Ca²⁺ channel function, impaired glucose-tolerance is not a frequent side effect of Ca²⁺ channel blockers in clinical practice. The reason for the absence of side effects in other tissues can be explained by the lower bioavailability in these tissues, by alternatively spliced channel subunits conferring different drug sensitivity (see above) and by the existence of different L-type channel isoforms, which differ in drug sensitivity. Ca_v1.2 channels, the major L-type channel isoform in vascular smooth muscle and cardiac ventricular muscle, are more sensitive to Ca²⁺ channel blockers than Ca_v1.3 and Ca_v1.4 isoforms, the major isoforms expressed in cochlear inner hair cells and retinal photoreceptors, respectively. Notably Ca_v1.3 channels also control cardiac pacemaking in the sinoatrial node but do not contribute to cardiac inotropy and arterial tone. This may provide new opportunities to develop isoform-selective blockers as bradycardic agents lacking negative inotropic actions.

Other Pharmacological Actions of Ca²⁺ Antagonists

Adult dopamin-containing neurons in the substantia nigra rely on Ca_v1.3 channels as pacemaker channels. It appears that the resulting enhanced Ca²⁺ load renders these channels more susceptible to neurotoxic effects and neurodegeneration as observed in Parkinson's disease. Preclinical evidence suggests that block of these with dihydropyridines causes a switch to a Ca_v1.3-independent pacemaker and protects these neurons from neurotoxicity.

Some DHPs (such as nifedipine) and verapamil inhibit ►p-glycoprotein-mediated drug transport. P-glycoprotein is a drug efflux pump that can confer multidrug resistance to tumor cells. Structural analogues with potent p-glycoprotein but weak Ca²⁺ channel blocking activity were therefore developed but are of no clinical benefit for the treatment of cancer. However, inhibition of transport (and excretion) of other p-glycoprotein substrates, such as digoxin, explains the decrease of their body clearance by Ca²⁺ channel blockers.

In vitro nifedipine inhibits proliferation of colon cancer cells with a DNA mismatch repair defect that are resistant to 5-fluorouracil. Whether this also

translates into clinical efficacy in such tumors remains to be determined.

Nimodipine, but not other DHPs, is also a potent inhibitor of nucleoside transport with actions similar to known nucleoside transport inhibitors such as dipyrindamol. It is likely that this mechanism also contributes to the potent vasodilating properties of this DHP.

BAYK8644 is a DHP with Ca^{2+} channel activating properties. Although some therapeutic effects can be envisaged for such drugs (such as stimulation of glucose-dependent insulin secretion, positive inotropy), severe side effects are also predicted from animal studies (dystonic neurobehavioral syndrome, hypertension, arrhythmias), which currently prevents their clinical development.

- Antihypertensive Drugs
- Voltage-dependent Ca^{2+} Channels

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Ca^{2+} -dependent Adhesion Protein Plakoglobin

- Cadherins/Catenins

Ca^{2+} -dependent Cysteine Proteases

- Calpains

Ca^{2+} -induced Ca^{2+} Release

A regenerative process whereby an intracellular Ca^{2+} channel (IP_3 receptor or Ryanodine receptor) is itself stimulated by Ca^{2+} , allowing thereby Ca^{2+} to promote its own release from intracellular stores.

- IP_3 Receptors
- Ryanodine Receptors

Ca^{2+} Receptor

- Ca^{2+} -sensing Receptor

Ca^{2+} Release Channel

Ryanodine Receptor.

- Table appendix: Membrane Transport Proteins

Ca^{2+} -sensing Receptor

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Synonyms

Calcium receptor; Extracellular calcium-sensing receptor (CaSR)

Definition

The extracellular calcium Ca_0^{2+} -sensing receptor plays a central role in maintaining a nearly constant level of extracellular calcium by sensing small changes in Ca_0^{2+} and directly and/or indirectly altering the translocation of calcium ions into or out of the extracellular fluid so as to normalize Ca_0^{2+} . Changes in the level of expression and/or function of the CaR reset the level of Ca_0^{2+} . Recently developed activators (calcimimetics)

upon ligand binding. There are additional calcium-binding site(s) in the receptor's transmembrane domains (TMDs), since a "headless" receptor lacking the entire ECD still responds to polyvalent cations [2]. The CaR's extracellular domain is extensively N-glycosylated, which is essential for efficient cell surface expression.

Following the ECD is a 250 amino acid, **▶TMD** motif characteristic of the GPCRs, which is followed by a carboxy-terminal tail of ~200 residues. Upon ligand binding, closure of the dimeric CaR's Venus flytrap motifs is thought to change the relative positions of the helices, thereby activating intracellular signaling. The CaR's C-tail contains several functionally important structural motifs, including protein kinase A and protein kinase C phosphorylation sites. Phosphorylation of the latter inhibits receptor-mediated activation of **▶phospholipase C (PLC)**. The C-tail interacts with filamin, an actin-binding protein that also serves as a scaffold for numerous other proteins, including components of the mitogen-activated protein kinase (**▶MAPK**) family, potassium channels and caveolin-1 [3]. The latter binds cholesterol and numerous signaling molecules (e.g., **▶G proteins**) and serves as a structural framework for plasma membrane caveolae. The latter are flask-like invaginations of the plasma membrane that contain numerous signaling molecules, presumably facilitating CaR signaling, and also participate in endocytosis, exocytosis, and transcytosis. Thus the CaR interacts with two proteins that may be key participants in its capacity to regulate intracellular signaling by virtue of their capacity to bind to and organize signaling networks at key cellular locations.

The CaR undergoes regulation at several levels. Protein kinase C-mediated phosphorylation of the receptor's C-terminal tail inhibits CaR-mediated activation of PLC, as noted above. Although downregulation of the CaR (e.g., due to uncoupling of the receptor from its G protein or internalization into the cell) probably does not represent a major form of regulation in vivo, it can take place in heterologous expression systems. In cells in which the constant availability of functioning receptor is essential for monitoring Ca_0^{2+} , filamin's capacity to inhibit agonist-induced internalization – presumably by tethering the CaR to the cytoskeleton – probably promotes resistance to desensitization by ambient levels of Ca_0^{2+} . Several factors regulate the expression level of the CaR gene [3]. Both high Ca_0^{2+} and $1,25(\text{OH})_2$ vitamin D_3 upregulate the receptor in some cells, the latter doing so via a regulatory element upstream of the CaR gene. Both of these factors inhibit parathyroid function, and likely do so, in part, by upregulating expression of the CaR, which mediates suppression of **▶parathyroid hormone (PTH)** secretion, PTH synthesis, and parathyroid cellular proliferation by high Ca_0^{2+} . Additional factors increasing CaR gene expression are interleukin (IL)-1 β and IL-6.

In addition to extracellular calcium, numerous other factors activate the CaR [3]. Almost any polycationic atom or molecule activates the receptor. The divalent cations magnesium, strontium, and barium, as well as some divalent heavy metals activate the CaR at millimolar levels. Of these, only Mg^{2+} is normally present in the ECF at concentrations capable of modulating the receptor's biological activity; indeed the CaR is thought to participate in "setting" Mg_0^{2+} . Trivalent cations of the lanthanide series, e.g., La^{3+} and Gd^{3+} , are also effective CaR agonists at micromolar concentrations. Various organic polycations are likewise potent stimulators of the CaR, including spermine, neomycin and other aminoglycoside antibiotics, protamine, and polyarginine.

Two other classes of molecules, in addition to polycations, modulate the CaR's activity. Some amino acids at millimolar concentrations, particularly aromatic amino acids, activate the receptor at levels of Ca_0^{2+} above 1 mM, i.e., they act as allosteric modulators [3]. The amino acid analogue, glutathione, is a much more potent activator of the receptor, acting at nanomolar concentrations, although whether it does so in vivo is not known. The amino acids have a predicted binding site close to the Ca^{2+} -binding site in the crevice between the two lobes of each CaR monomer, potentially explaining the receptor's allosteric regulation by amino acids. Another class of molecules, known as **▶calcimimetics**, is also allosteric activator of the CaR, acting at nanomolar to micromolar concentrations [4]. The early generations of these drugs, which are useful in treating various forms of hyperparathyroidism (see below), were phenylalkylamines. They bind to a site distinct from that of the amino acids, which is thought to involve the receptor's TMDs, with the amino group of the drugs binding to glutamate 837, just at the outer end of TM7. Other factors influencing the receptor are increases in ionic strength, which reduce the CaR's apparent affinity for Ca_0^{2+} , and elevation in pH, which has the opposite action.

Like other GPCRs, the CaR regulates numerous signaling pathways, in many cases via the G proteins, $\text{G}_{q/11}$ and G_i [1] [3]. The receptor activates PLA_2 (via the MAPK, ERK1/2, and/or calmodulin-dependent kinases), PLC (via $\text{G}_{q/11}$), and PLD (via $\text{G}_{12/13}$), and inhibits cAMP formation by inhibiting adenylate cyclase (via G_i or calcium-inhibitable forms of the enzyme). The receptor also activates the lipid kinases, PI3-kinase and PI4-kinase (by a rho-dependent pathway in the latter case), which phosphorylate polyphosphoinositides that are themselves mediators or can be cleaved to biologically active products (e.g., IP_3). Finally, the CaR activates multiple MAPKs, including extracellular-regulated kinase (ERK1/2), p38 MAPK, and Jun N-terminal kinase (JNK). While the precise signaling pathways that the CaR utilizes to regulate the

biological functions of some cells are known, much remains to be learned in this regard.

The CaR regulates numerous biological processes, including the expression of various genes (e.g., PTH); the secretion of hormones (PTH and calcitonin), cytokines (MCP-1), and calcium (e.g., into breast milk); the activities of channels (potassium channels) and transporters (aquaporin-2); cellular shape, motility (of macrophages), and migration; cellular adhesion (of hematopoietic stem cells); and cellular proliferation (of colonocytes), differentiation (of keratinocytes), and apoptosis (of H-500 leydig cancer cells) [3].

Role of the CaR in Ca₀²⁺ homeostasis. The CaR plays a central role in orchestrating the responses of the homeostatic system governing extracellular calcium homeostasis to alterations in Ca₀²⁺. In response to hypocalcemia, for instance, the CaR in the parathyroid gland senses the change in Ca₀²⁺ and responds with increases in the biosynthesis and secretion of PTH as well as enhanced parathyroid cellular proliferation [3]. PTH acts on the kidney to increase tubular reabsorption of calcium and enhance the synthesis of the active vitamin D metabolite, 1,25(OH)₂D₃. The latter increases intestinal calcium absorption and, together with PTH, stimulates skeletal release of calcium. Increased influx of calcium into the ECF from intestine and bone, coupled with reduced excretion of calcium by the kidney in most cases effectively normalizes Ca₀²⁺. In addition to being present in the parathyroid, however, the CaR is also expressed in the kidney, where it enhances renal excretion of calcium in response to hypercalcemia and may inhibit the synthesis of 1,25(OH)₂D₃. The receptor is likewise expressed in intestine and bone, where it may carry out physiologically relevant actions [3]. In bone, for example, raising Ca₀²⁺ stimulates the activity of bone-forming osteoblasts and inhibits the activity of bone-resorbing osteoclasts, actions that would promote a homeostatically appropriate lowering of Ca₀²⁺. Available evidence, however, is conflicting as to whether the CaR or some other Ca₀²⁺-sensing receptor mediates these skeletal actions of Ca₀²⁺.

Disorders with reduced sensitivity of the CaR to Ca₀²⁺. Two conditions result from heterozygous or homozygous inactivation of the CaR, ►familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT), Familial hypocalciuric hypercalcemia and neonatal severe hyper parathyroidism; two forms of PTH-dependent hypercalcemia that result, respectively, from the presence of one or two CaR alleles bearing inactivating mutations, respectively (Fig. 2) [1–3]. The former manifests as mild to moderate hypercalcemia without the usual symptoms and complications of hypercalcemia. In addition to exhibiting PTH-dependent hypercalcemia, these patients excrete normal or frankly low amounts of calcium in their urine despite being

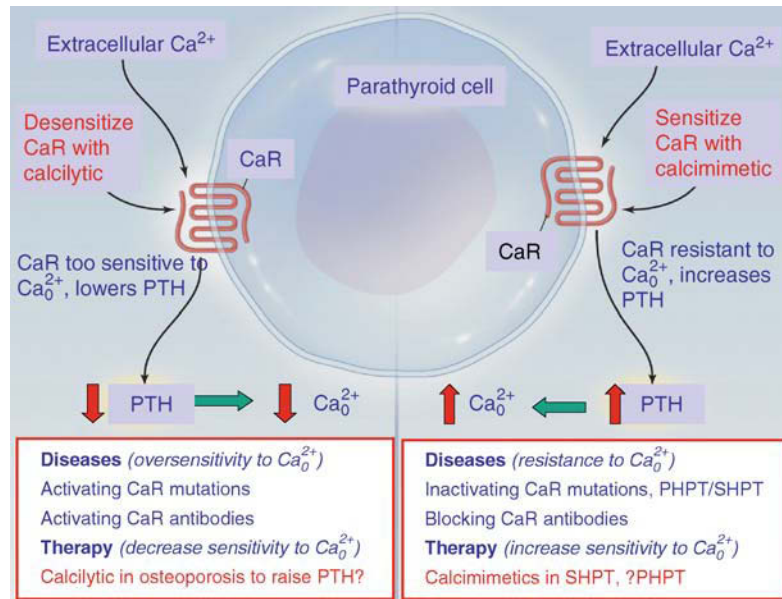
hypercalcemic, which in other settings would be expected to produce hypercalciuria. Because of their benign clinical course and tendency to rapid recurrence of hypercalcemia unless all parathyroid tissue is removed, most FHH patients should simply be followed untreated. NSHPT is a severe form of hyperparathyroidism encountered in the first 6 months of life often complicated by excessive, PTH-mediated bone loss, fractures and marked elevation in serum calcium concentration. These infants require urgent medical treatment of their hypercalcemia and in very severe cases may require total parathyroidectomy to restore normocalcemia and heal their bone disease.

Well over a hundred inactivating mutations of the CaR have been described, including missense, splice site, deletion, insertion, and deletion mutations. These mutations reduce the activity of the CaR by interfering with its biosynthesis and cell surface expression, reducing its affinity for Ca₀²⁺ and/or interfering with signal transduction. In addition to the reduction in CaR signaling in FHH resulting from the loss of one normal CaR allele, some mutant receptors exert a dominant negative action on the normal CaR when present within wild type-mutant heterodimers.

The parathyroid glands in FHH are reset to maintain a higher than normal serum calcium concentration owing to impaired suppression of PTH release in the face of hypercalcemia (e.g., “resistance” to Ca₀²⁺) (Fig. 2). Similarly the kidneys show a reduced calciuric response to hypercalcemia, which contributes to the hypercalcemia by promoting inappropriately reabsorption of calcium. Mouse models of FHH and NSHPT result from targeted inactivation of one or both CaR alleles, respectively [1,3]. These animals have provided valuable insights into the alterations in tissue function resulting from loss of the receptor.

Several patients have exhibited a clinical picture resembling FHH, but no CaR mutations could be identified. These individuals also exhibited various forms of autoimmunity (e.g., antithyroid antibodies) and harbored anti-CaR antibodies that reduced the high Ca₀²⁺-evoked stimulation of MAPK and PLC in cells transfected with the wild type receptor. Thus both antireceptor antibodies and mutations in the CaR can render the receptor resistant to activation by Ca₀²⁺, producing a clinical picture of mild, PTH-dependent hypocalciuric hypercalcemia [3].

A common cause of PTH-dependent hypercalcemia results from benign, or occasionally malignant, enlargement of one or more parathyroid glands, a condition known as ►primary hyperparathyroidism (PHPT). Although many patients with PHPT present in an asymptomatic state that does not require medical intervention, some are afflicted with excess bone loss, kidney stones, or other complications. If patients are



Ca^{2+} -sensing Receptor. Figure 2 Schematic illustration of the diseases of the CaR and their treatments with calcimimetics or calcilytics. The CaR becomes resistant to extracellular calcium in diseases resulting from inactivating mutations (familial hypocalciuric hypercalcemia /neonatal severe hyperparathyroidism; FHH/NSHPT) or antibodies to the CaR as well as in primary hyperparathyroidism. This leads to “resetting” of the serum calcium concentration upward as a result of failure of the parathyroid cells to appropriately suppress PTH secretion in response to hypercalcemia. The parathyroid glands can be “resensitized” to Ca_0^{2+} with a calcimimetic, thereby resetting the level of calcium back toward normal. These drugs are only currently approved, however, for use in secondary hyperparathyroidism (SHPT) in patients with end stage renal failure as a means of lowering the elevated parathyroid hormone (PTH) levels frequently encountered in this condition to the desired concentration. In contrast, activating mutations or antibodies render the receptor too sensitive to Ca_0^{2+} , resetting calcium in the blood to a lower level. A calcilytic would likely be useful in this setting to raise the level of blood calcium toward normal, but this class of drugs has not been approved for this use. Another application of calcilytic currently under investigation is to stimulate endogenous PTH release by “tricking” the parathyroid glands into sensing hypocalcemia, thereby releasing a pulse of PTH, as a potential anabolic therapy for osteoporosis, analogous to the daily injections of PTH currently being used for this purpose. Reproduced with permission from Stewart AF (2004) *N Engl J Med* 351: 324–326.

symptomatic or show signs of end organ damage, they should undergo removal of the enlarged, neoplastic parathyroid gland(s), although the newly developed calcimimetic CaR activators may in the future represent an effective form of medical therapy. There is a reduced level of CaR expression in pathological parathyroid glands from patients with PHPT, although it has not been established that reduced CaR expression causes the resistance of the parathyroid to Ca_0^{2+} in PHPT. Indeed, there can also be reductions in components of the parathyroid CaR “signaling complex,” i.e., $\text{G}_{q/11}$ and/or caveolin-1, which may also contribute. The parathyroid glands of patients with the severe ▶**secondary hyperparathyroidism (SHPT)** observed in some patients with renal failure also exhibit reduced CaR expression, although the biological significance of this change is still under investigation.

Disorders with enhanced sensitivity of the CaR to Ca_0^{2+} . In contrast to inactivating mutations of the CaR, activating antibodies produce a syndrome of ▶**autosomal**

dominant hypocalcemia (ADH) accompanied by absolute or relative hypercalciuria (e.g., inappropriately high in the face of hypocalcemia) (Fig. 2) [3]. This clinical syndrome is generally benign, although some patients experience seizures or other manifestations of severe hypocalcemia. Only symptomatic patients should be treated with calcium and vitamin D, and serum calcium should be raised only enough to ameliorate symptoms, as these patients are prone to develop severe hypercalciuria with attendant risk of kidney stones or renal calcification during treatment.

Some patients with reduced or absent parathyroid function, e.g., primary hypoparathyroidism, harbor anti-CaR antibodies (Fig. 2) [4]. In two patients, the antibodies were shown to activate the CaR as assessed by stimulation of MAPK and PLC. Thus, analogous to activating mutations of the CaR, anti-CaR antibodies can increase the sensitivity of the receptor to Ca_0^{2+} , thereby resetting parathyroid and kidney to maintain hypocalcemia.

Drugs

Calcimimetics, potent allosteric activators of the CaR, provide an effective medical therapy for certain forms of hyperparathyroidism (Fig. 2) [4]. In patients receiving dialysis treatment for end stage renal disease (ESRD), markedly elevated PTH levels can promote excessive loss of calcium from the skeleton leading to bone pain and increased risk of fracture. Chronically elevated PTH levels as well as phosphate retention also increase the risk of cardiovascular disease, particularly myocardial infarction. The US National Kidney Foundation has published guidelines for therapeutic targets expected to reduce cardiovascular risk and other complications of ESRD. These include serum calcium and phosphate concentrations close to the normal range (Ca²⁺, 2.1–2.4 mmol/L; phosphate 1.12–1.76 mmol/L) and PTH levels about 3-fold to 5-fold above normal 150–300 pg/ml (levels of PTH thought to maintain normal bone turnover in ESRD). In a substantial number of patients, PTH levels remain excessively elevated despite therapy with conventional forms of therapy, i.e., active metabolites of vitamin D and phosphate binders.

It is in this group of patients that the calcimimetics promised an effective means of lowering PTH levels further [4] [5]. Clinical trials have shown that the calcimimetic, Cinacalcet (also known as Sensipar in the US and Mimpara in Europe), which has been approved by the US Food and Drug Administration, lowers PTH to the target range in more than 50% of patients unresponsive to conventional therapies, while maintaining serum calcium and phosphate close to normal range in most patients. Unexpectedly, Cinacalcet also lowered the calcium–phosphate product (serum calcium multiplied by serum phosphate) by about 15%, a response expected to reduce cardiovascular complications. A small meta-analysis pooling data from several clinical trials with Cinacalcet showed that it reduced risk of fracture, parathyroidectomy, and hospitalization for cardiovascular complications.

Although not yet approved by the FDA for use in PHPT, clinical trials have shown that Cinacalcet restores normocalcemia in nearly three quarters of patients with this condition [3]. In parathyroid cancer, a rare form of PHPT, available data indicate that it lowers serum calcium concentration in about 60% of patients, although generally not to normal levels. Whether by reducing morbidity or mortality associated with severe hypercalcemia, it lengthens life or promotes improved quality of life in this latter setting remains to be determined.

► **Calcilytics**, in contrast to calcimimetics, block rather than mimic the actions of Ca₀²⁺ on the CaR. Administering a rapidly acting calcilytic causes a transient increase in circulating PTH by “tricking” the parathyroid glands into sensing hypocalcemia (Fig. 2) [4]. Ongoing studies are exploring the possible utility of calcilytics as an oral form of treatment for osteoporosis

that mimics the increase in circulating PTH levels resulting from administration of PTH by injection – a known anabolic treatment for osteoporosis.

► CaR

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Ca²⁺ Sparks

Ca²⁺ sparks are localized and transient Ca²⁺ release observed recurrently in muscle cells and skinned fibres. A Ca²⁺ spark is considered to be the elementary process of Ca²⁺ release in situ from one to a few ryanodine receptors.

► Ryanodine Receptor

Ca²⁺ Spikes

Short (typically lasting no more than a few seconds) increases in cytosolic Ca²⁺ concentration that periodically interrupts the stable resting level. Many Ca²⁺ signals are delivered to cells as frequency-coded Ca²⁺ spikes.

► IP₃ Receptors

Ca²⁺ Transient

A typically prolonged transient increase in intracellular Ca²⁺ concentration, which is detected by a Ca²⁺ indicator. The increased Ca²⁺ levels are due to Ca²⁺

release from intracellular Ca^{2+} -stores and to Ca^{2+} influx from the external medium.

- ▶ IP_3 Receptor
- ▶ Ryanodine Receptor
- ▶ Non-selective Cations Channels
- ▶ TRP Channels

Ca²⁺ Waves

Local and transient Ca^{2+} increases that propagate throughout the cytosol of individual cells in the form of waves. Ca^{2+} waves are generated by a positive feedback activation of Ca^{2+} release from the intracellular Ca^{2+} stores through ryanodine receptors or inositol IP_3 receptors.

- ▶ IP_3 Receptor
- ▶ Ryanodine Receptor

Cachexia

Cachexia refers to a physical wasting due to loss of muscle and fat. Cachexia is often found in end-stage cancer patients but is also caused by autoimmune disorders or by infectious diseases such as AIDS and tuberculosis.

- ▶ Tumor Necrosis Factor
- ▶ TOR Signalling

Cachexin

- ▶ Tumor Necrosis Factor (TNF)

Cadherins

- ▶ Cadherins/Catenins
- ▶ Table appendix: Adhesion Molecules

Cadherins/Catenins

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Synonyms

Calcium-dependent adhesion protein/plakoglobin

Definition

Cadherins (Calcium-dependent adhesion proteins) are transmembrane proteins, which consist of an extracellular domain composed of cadherin-repeats, a transmembrane domain, and a cytoplasmic domain that interacts with catenins and/or other cytoplasmic proteins.

Catenins are defined as cytoplasmic interaction partners of cadherins that form a chain of proteins ("catena," latin for chain), which connects cadherins to the actin cytoskeleton.

Basic Characteristics

Structural Characteristics

Cadherins are a superfamily of Ca^{2+} -sensitive cell–cell adhesion molecules, which cause [▶homophilic cell interactions](#). Cadherins can be divided into different subfamilies, namely, classical cadherins, desmosomal cadherins, protocadherins, and nonconventional cadherins (7TM cadherins, T-cadherin, FAT). Classical cadherins are often denoted by a prefix reflecting their principal expression domains; e.g., E is epithelial, N is neuronal, and P is placental. However, this classification is not stringent, as for instance E-cadherin can also be found in certain neuronal tissues, and N-cadherin is also found in epithelial cells. Among the desmosomal cadherins, two subfamilies can be distinguished: the desmocollins 1–3 and the desmogleins 1–4.

The extracellular domain of cadherins consists of a variable number of a repeated sequence of about 110 amino acids. This sequence is termed the "cadherin repeat" and resembles in overall structure, but not in sequence, the Ig like domains. The cadherin repeat is the characteristic motive common to all members of the cadherin superfamily. Classical and desmosomal cadherins contain five cadherin repeats, but as many as 34 repeats have been found in the FAT cadherin (see below). Cadherins are calcium-dependent cell adhesion molecules, which means that removal of Ca^{2+} , e.g., by chelating agents such as EDTA, leads to loss of cadherin function. The Ca^{2+} -binding pockets are made up of amino acids from two consecutive cadherin repeats, which form a characteristic tertiary structure to coordinate a single Ca^{2+} -ion [1].

The classical cadherins are translated as precursor because they are N-terminally cleaved to reveal the mature proteins. This processing is required to activate the cell adhesion function of cadherins. Cadherins interact in trans (i.e., from opposite cells) via the most N-terminal cadherin repeats. A short amino acid sequence within this repeat, histidine–alanine–valine (HAV), has been implicated in mediating cell–cell contacts as HAV peptides can disrupt cadherin-dependent cell adhesion. Besides the trans-interactions of cadherins, the extracellular domains are also capable of forming cis-dimers through lateral amino acid contacts between cadherin molecules on one cell. This dimerization again mainly involves the first cadherin repeat. A zipper model based on the pattern of alternating cis- and trans-dimers [1] for the adhesive interactions has been proposed.

Several nonconventional cadherins that contain cadherin repeats have been described but they have specific features not found in the classical cadherins [1]. The cadherin Flamingo, originally detected in *Drosophila*, contains seven transmembrane segments and in this respect resembles G protein-coupled receptors. The extracellular domain of Flamingo and its mammalian homologs is composed of cadherin repeats as well as EGF-like and laminin motifs. The seven transmembrane span cadherins have a role in homotypic cell interactions and in the establishment of cell polarity. The FAT-related cadherins are characterized by a large number of cadherin repeats (34 in FAT and 27 in dachsous). Their cytoplasmic domains can bind to catenins. T- (=truncated-)cadherin differs from other cadherins in that it has no transmembrane domain but is attached to the cell membrane via a ►glycosylphosphatidylinositol anchor.

Cytoplasmic Interactions of Cadherins

The cytoplasmic domains of cadherins bind to various proteins. The most C-terminal portion of classical cadherins directly associates with either β -catenin or the structurally related γ -catenin (more commonly called plakoglobin). β -Catenin/plakoglobin in turn binds to α -catenin, which is related to the cytoskeletal protein vinculin and associates with actin-binding proteins. Thus, β - and α -catenin provide a link of cadherins to the actin cytoskeleton, although recent evidence indicates that this is indirect, i.e., α -catenin interacts with actin and β -catenin in separate complexes. Another catenin, p120^{ctn} binds to the cytoplasmic juxtamembrane domain of cadherins and appears to be involved in the cis-dimerization and clustering of cadherins. Based on their structure, β -catenin, plakoglobin, and p120^{ctn} belong to a protein superfamily, called the armadillo repeat family [2]. These proteins contain repeats of about 40 amino acid that were initially identified in armadillo, the *Drosophila* homolog of β -catenin. The armadillo repeats are built up of three α -helices that form a

superhelix. The number of repeats varies between different members of the family; for instance, β -catenin contains 12 consecutive armadillo repeats. More distantly related members of the armadillo family include nonjunctional proteins, such as the importins, which are involved in nuclear protein import, and the tumor suppressor APC. p120^{ctn} is the founding member of a subfamily of armadillo proteins that include the plakophilins 1–3, p0071, ARVCF, and the nervous system specific δ -catenin; the latter two are binding to the same region of cadherins as p120^{ctn}. Interestingly, the p120^{ctn} related proteins are also found in the cell nucleus. p120^{ctn} binds to the transcription factor Kaiso relieving its repressor activity, and plakophilin 2 is associated with RNA polymerase III complexes. Loss of p120^{ctn} function in mice leads to activation of NF κ B signaling and inflammation. The desmosomal cadherins also associate with plakoglobin but not with β -catenin, and in addition they bind to plakophilins. Plakoglobin and plakophilins connect desmosomal cadherins to the desmosome specific component ►desmoplakin, which links the complexes to the intermediate filament network (mainly ►cyto-keratins in epithelial cells).

The cytoplasmic domains of protocadherins are unrelated to those of classical cadherins. They do not bind catenins and it is not clear whether they are associated with the cytoskeleton [1]. Some protocadherins interact with the c-►src-related kinase ►Fyn, indicating a role in signal transduction (see below).

Gene Organization

Several classical cadherin genes (e.g., E-, P-, VE-cadherins and others) are found in a cluster on human chromosome 16 or on the syntenic mouse chromosome 8. The gene clustering is even more pronounced in the case of the desmosomal cadherins, which are all located on human chromosome 18q12 in relatively close vicinity to each other. Interestingly, the desmocollin and desmoglein genes (termed *DSC* and *DSG*) form two subclusters that have opposite transcriptional orientation. Protocadherins show a particularly striking genome organization. Subfamilies of protocadherins can be defined as those that share a common intracellular domain but differ in their extracellular domains [1].

Functional Characteristics

Classical and desmosomal cadherins are constituents of different types of intercellular junctions. E-cadherin, the classical cadherin of epithelial cells, is part of the adherens junction (zonula adherens), which is attached to a belt of actin via the catenins. As the name says, desmosomal cadherins are part of the desmosomes, which are rivet-like structures that make focal connections between cells. Desmosomes are characterized by a

prominent intracellular plaque structure, which serves as an attachment point for intermediate filaments.

Functional studies show the adhesive role of cadherins [1]. For example, cell–cell adhesion *in vitro* can be blocked by treatment of cultured cells with anti-cadherin antibodies, resulting in dissociation of the cell monolayer. Conversely, forced expression of cadherins by cDNA transfection in cells lacking these molecules leads to establishment of intercellular contacts. Thus, cadherins act as an adhesive glue that efficiently holds cells together. However, they may also have signaling function, as several *in vitro* studies show that cadherins influence differentiation and growth of cells. *In vitro*, cadherins can mediate the ►[sorting out](#) of cells, i.e., cells transfected with different cadherins separate from each other and forming homotypic aggregates. Accordingly, one of the main functions of cadherins might be in the delineation of tissue boundaries. Gene knockout experiments have revealed essential and cell-type specific functions of cadherins *in vivo*. The knockout of the E-cadherin gene results in dissociation of cells of blastocyst-stage mouse embryos. The α -catenin knockout has a similar phenotype, while loss of plakoglobin leads to defects in cell adhesion of heart muscle cells. The knockout of β -catenin does not affect cell junctions but rather has a Wnt signaling phenotype (see below), which is manifested at early stages of embryonic development. Apparently, plakoglobin, which is also present in the affected cells, can overtake the adhesive function of β -catenin under these circumstances. The mutation of desmosomal cadherins frequently leads to the disruption of skin layers. These phenotypes resemble those of the ►[paphyphigus blistering diseases](#) in humans, which are caused by autoantibodies against desmocolins and desmoplakins.

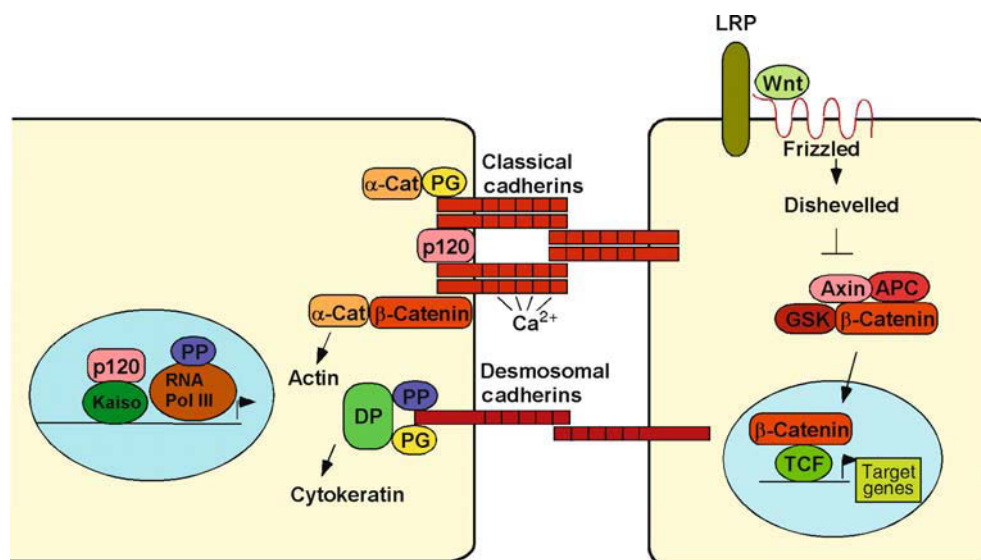
E-cadherin appears to play a major role in cancer as its expression is frequently downregulated in dedifferentiated, metastasizing tumors as well as during ►[epithelial-mesenchymal transitions](#) in embryonic development. E-cadherin gene expression is repressed by transcription factors of the Snail/Slug and ZEB1/ZEB2 families, as well as by Twist and E12/47 transcriptional repressors, which all bind to specific DNA elements in the E-cadherin promoter. E-cadherin inhibits invasion of tumor cells in *in vitro* systems and prevents tumor progression *in vivo* animal models. Moreover, loss of function mutations of the E-cadherin gene occurs in gastric carcinomas and certain types of breast tumors. The function of cadherins can be modulated by signaling pathways involving tyrosine kinases, which disrupt cell contacts, as well as by RhoA family GTPases. Conversely, E-cadherin can modulate the activity of these GTPases, although these signaling events have not been clearly defined in molecular terms so far [1].

It is not clear whether protocadherins are true cell adhesion molecules since some of them show only moderate activity in classical cell aggregation assays [1]. However, in *Xenopus* embryos, expression of a dominant-negative mutant of NF-protocadherin leads to disruption of the embryonic ectoderm, indicating a role of this protocadherin in maintaining tissue integrity. Biochemical and embryological evidence indicates that protocadherins also have a role in intercellular signaling. A subfamily of protocadherins, termed CNRs (cadherin-related neuronal receptors), associates with ►[Fyn](#), a cytoplasmic tyrosine kinase related to ►[c-src](#). The extracellular matrix protein ►[reelin](#) binds to these CNRs and activates the Fyn kinase, which leads to phosphorylation of a downstream signaling protein, mDAB. This pathway appears to be important for certain steps in cortical neuron development, as antibodies to the reelin-binding domain of CNRs perturb the arrangement of cortical neurons. Since there are so many members of the protocadherin family, which are mostly expressed in the nervous system, it has been speculated that protocadherins play discrete roles in setting up neuronal networks [1].

In *Drosophila*, *Fat* functions as a ►[tumor suppressor gene](#) and *dachsous* is involved in thorax, leg, and wing development. Several human and mouse *FAT* homologs have been identified. *FAT1* regulates actin filaments, and the *Fat1* knockout leads to defects in glomerular slit formation [3].

►[β-Catenin in the Wnt pathway](#)

Besides its role in cell adhesion, β -catenin has an important function as a central signal transduction component in the Wnt pathway [4, 5]. Wnts are a family of secreted glycoproteins that regulate a variety of developmental processes. Binding of Wnts to Frizzled receptors, which are ►[seven transmembrane span proteins](#), induces stabilization of β -catenin. This pool of β -catenin is not associated with cadherins but accumulates in the cytoplasm and eventually enters the nucleus, where it teams up with transcription factors of the TCF family. TCFs bind to specific DNA sequences in Wnt-target promoters via an ►[HMG box](#) but lack transactivation domains. In the absence of β -catenin, TCFs behave as transcriptional silencers, in part, because they bind to diverse transcriptional repressors. β -Catenin lacks DNA-binding activity but contains strong transactivating sequences in its N- and C-terminal domains. Thus, when β -catenin binds to TCFs a bipartite transcription factor is formed in which DNA-binding and transactivation domains reside on separate molecules. The TCF/ β -catenin complexes can activate specific Wnt-target genes involved in determining cell fate and differentiation and inducing cancer (see below). For a list of Wnt-target genes see ►www.stanford.edu/~rnusse/wntwindow.html.



Cadherins/Catenins. Figure 1 Overview of cadherin-mediated cell adhesion and the Wnt signaling pathway. Schematic representation of classical and desmosomal cadherins showing extracellular cadherin repeats and Ca²⁺-binding sites, and cytoplasmic interaction partners (the latter being depicted in the left cell only, which also shows association of p120^{cas} and plakophilin 2 with transcriptional regulators in the nucleus). The hypothetical cadherin zipper, which is made up of consecutive cis- and trans-dimers is indicated [1]. The basic outline of the Wnt pathway is shown in the right cell. LRP (lipoprotein receptor-related protein) is a coreceptor for Wnts. PG, plakoglobin, PP, plakophilin.

In the absence of Wnts, cytoplasmic “free” β-catenin is targeted for degradation by a multiprotein complex containing the scaffold component axin or the related protein conductin, the ►**tumor suppressor APC** (adenomatous polyposis coli), and the serine/threonine kinases CK1 and GSK3β. When β-catenin binds to axin it becomes phosphorylated by CK1 and GSK3β. Hyperphosphorylated β-catenin is recognized by the ►**E3 ligase βTrCP/slimb**, a component of the ubiquitination machinery, ubiquitinated, and finally degraded in proteasomes [4]. APC has several β-catenin and axin-binding sites and may function by sequestering free β-catenin and delivering it to the axin complex [4]. Thus, APC acts as a safeguard to prevent aberrant accumulation of β-catenin. Mutations of APC occur in up to 80% of colorectal carcinomas and lead to the formation of truncated proteins that are no longer able to interact with axin/conductin and to induce degradation of β-catenin. Therefore, these mutations result in the stabilization of β-catenin and in the formation of constitutive TCF/β-catenin complexes, which activate transcription of oncogenic target genes in a Wnt-independent fashion. In some colorectal tumors, and more frequently in other tumor types (e.g., hepatoblastomas), stabilization of β-catenin occurs through mutations of the critical serine or threonine residues normally phosphorylated by GSK3β. Thus both genetically and functionally β-catenin behaves as an oncogene and the Wnt pathway has a major role in tumorigenesis (Fig. 1).

Drugs

There are several potential approaches for pharmacological interference with the cadherin/catenin system. Drugs that upregulate E-cadherin in tumors could be of potential benefit as part of a differentiation strategy to “normalize” epithelial cancers and to prevent metastasis formation, although such substances have not yet been described. In contrast, drugs that affect components of the Wnt pathway, e.g., by blocking the interaction of TCFs with β-catenin, have been identified and are candidates for interference with tumor growth [5].

►Table Appendix: Adhesion Molecules

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cADP-ribose

cADP-ribose (cyclic ADP-ribose) has been shown to trigger the release of calcium from the endoplasmic reticulum via ryanodine receptors (calcium release channels). Cyclic ADP-ribose is enzymatically formed and appears to act as a second messenger, which mediates a sustained increase in cytosolic calcium in various activated eucaryotic cells including T lymphocytes.

► [Ryanodine Receptor](#)

Calbindins

Calbindins are hormonally controlled by vitamin D and are expressed in the kidney, intestine, and brain.

► [Ca²⁺-binding Proteins](#)
 ► [Vitamin D](#)

Calcilytics

A class of drugs that blocks the activation of the Ca²⁺-sensing receptor by extracellular Ca²⁺.

► [Ca²⁺-sensing Receptor](#)

Calcimimetics

A class of allosteric activators of the Ca²⁺-sensing receptor that sensitizes the receptor to extracellular calcium and acts only in the presence but not in the absence of calcium. Calcimimetics can be used to treat various forms of hyperparathyroidism, although they are only approved for use in patients with end stage renal disease receiving dialysis treatment.

► [Ca²⁺-sensing Receptor](#)

Calcineurin

Calcium-dependent serine/threonine phosphatase (also known as protein phosphatase 2B or PP2B). Calcineurin is a heterodimer composed of a catalytic subunit (Calcineurin A) and a regulatory subunit that contains an autoinhibitory domain (Calcineurin B). The catalytic subunit also contains a calmodulin-binding domain. Binding of calcium to the regulatory subunit allows the binding of calmodulin to the catalytic subunit, resulting in displacement of the autoinhibitory domain and enzymatic activation. Calcineurin has many functions within eukaryote cells but is best known for its role in activating transcription of the interleukin 2 (IL-2) gene. IL-2 is required for activation of B- and T- cells and thus calcineurin inhibitors such as cyclosporin A and FK506, are potent immunosuppressants.

► [Protein Phosphatases](#)
 ► [NFAT Family of Transcription Factors](#)
 ► [TOR Signalling](#)
 ► [Immunosuppressive Agents](#)

Calcitonin

A peptide hormone rapidly inhibiting osteoclast activity. The relevance of calcitonin in human calcium homeostasis is not well understood. Calcitonin has been used for the treatment of osteoporosis, although due to the availability of more potent drugs with less side effects, and the lack of clear data on the anti-fracture efficacy of calcitonin, its clinical use has been steadily declining.

► [Bone Metabolism](#)

Calcitonin Gene Related Peptide

Calcitonin gene related peptide (CGRP) is one of the numerous peptides found in neurons and acting as cotransmitters. It is derived from the gene encoding calcitonin by alternative splicing of mRNA and by proteolytic processing of a precursor peptide. It is mainly found in sensory neurons of the central nervous system. Its prime target is the CGRP receptor, a member

of the family of G protein-coupled receptors. In contrast to calcitonin, which is involved in calcium homeostasis and bone remodeling, CGRP causes vasodilatation and vascular leakage. It is also expressed in C-fiber sensory neurons. It works as a stimulatory (pronociceptive) neurotransmitter when it is released centrally, and as a proinflammatory mediator when it is released peripherally. The central role of CGRP in primary headaches has led to a search for suitable antagonists of CGRP receptors.

► Analgesics

Calmodulin

Small ubiquitous calcium-binding protein. Calmodulin binds and regulates the activity of many protein targets involved in cellular signal transduction pathways mediated by calcium. Calmodulin is ranked among the most conserved proteins and plays a key role in many cellular processes.

- Ca^{2+} -binding Proteins
- Smooth Muscle Tone Regulation
- NFAT Family of Transcription Factors

Calpains

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Synonyms

Ca^{2+} -dependent cysteine proteases

Definition

Calpains are proteolytic ►enzymes with cysteine in the catalytic site. In general, calpains are found as intracellular ►proteases. These proteases are associated with many physiological basic processes of the cell.

Basic Characteristics

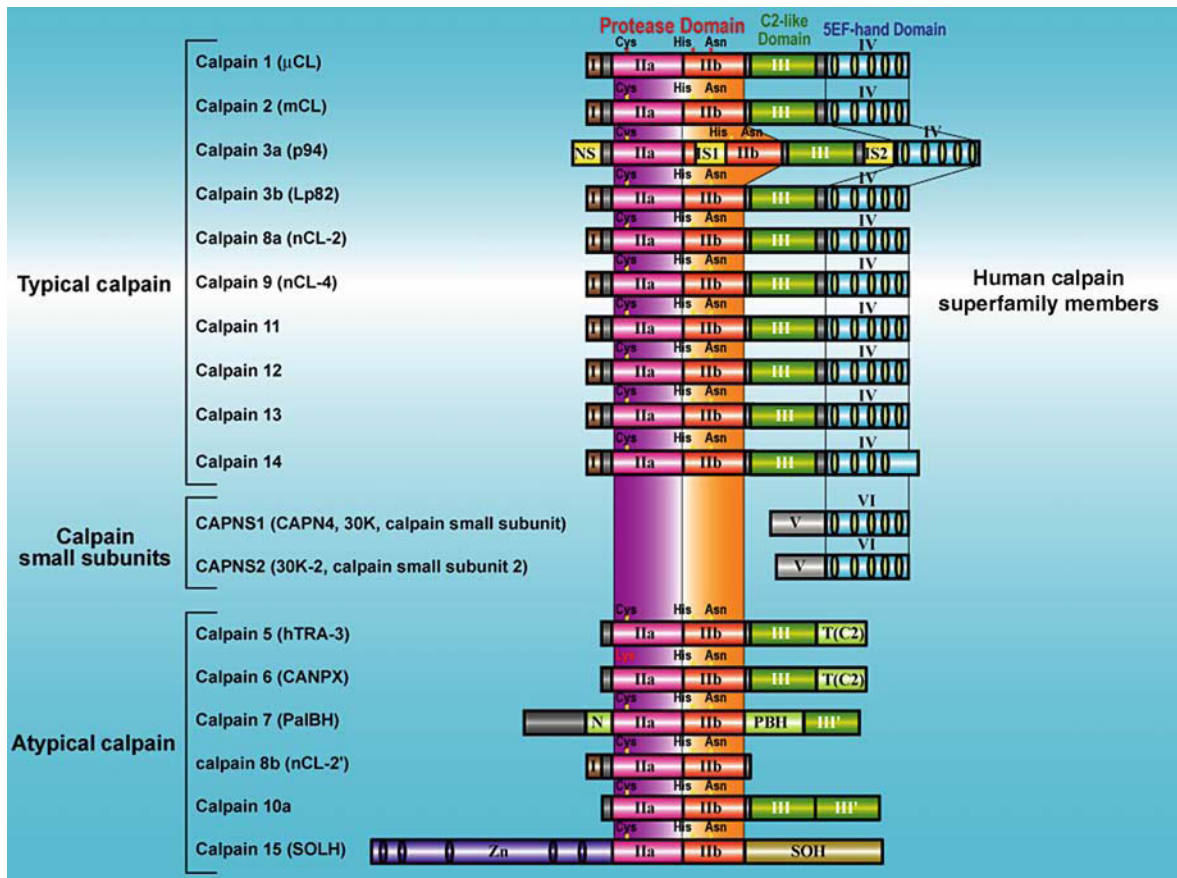
Calpain Superfamily

In higher organisms, calpain superfamily contains 16 independent genes that modulate cellular function. Out of them, 14 are Ca^{2+} -dependent cysteine proteases. The other two encode smaller regulatory proteins that

associate with some of the catalytic subunits to form heterodimeric proteases. Homologous of the calpain catalytic subunits are also present in lower organisms including nematodes, plants, flies, and yeasts. Substantial proportion of the calpains is not free in the cell cytoplasm, but associated with subcellular structures. Several calpain genes are expressed in specific tissues (calpains 3, 6, 8, 9, 11, and 12), whereas others are ubiquitously expressed (calpains 1, 2, 5, 7, 10, 13, and 15).

Calpain Structure

Two members of calpain superfamily, calpain 1 (μ -calpain) and 2 (m-calpain), have been extensively studied. Calpain 1 and 2 differ in their sensitivity to Ca^{2+} and each one of them contains two different polypeptide subunits existing as heterodimers (Fig. 1). The larger subunit (80 KDa) has catalytic activity, whereas the smaller (28 KDa) subunit has a regulatory function. Calpains 3, 8a, 9, 11, 12, and 13 also have the 80-KDa and 28-KDa subunits. The enzymatically active (large) subunit comprises up to four domains: domain I is the N-terminal anchoring α -helix domain, which is important for regulating the activity and dissociation of the subunit. This domain has no sequence homology with any polypeptide sequenced thus far, while sequence homology of domain I among different species (human, chicken, rat, and porcine) is 72–86%; domain II comprises the protease domain that contains the active site catalytic triad Cys105, His262, and Asn286. The ►active site Cys is in domain IIa, whereas the His and Asn that constitute the remainder of the catalytic triad are in domain IIb. Each of these IIa and IIb domains binds one atom of Ca^{2+} in a peptide loop consisting of 8 (IIa) or 9 (IIb) ►amino acids. Sequence Homology of domain II among different species is high, ranging from 85 to 93%; domain III is involved in binding phospholipids and in regulating calpain activity by its participating critical electrostatic interactions. This domain also contains two potential EF-hand Ca^{2+} -binding sequences, one at the domain II/III boundary, and the other at the domain III/IV boundary; and domain IV, also called as the penta-EF-hand domain (an EF-hand unit consists of two peptide helix connected by a Ca^{2+} -binding loop), is involved in dimerization of the 80-KDa and 28-KDa subunits. Sequence homology among the species ranges from 65 to 93% for this domain. The 28-KDa regulatory subunit of calpain 1, 2, and 9 consists of two domains: domain V is the N-terminal, glycine-rich, hydrophobic domain; and domain VI, the penta-EF-hand domain, which is similar to domain IV of the catalytic subunit. The 80-KDa subunit of the other calpains (3, 8a, 11, 12, and 13) does not interact with the 28-KDa subunit, although having domain IV. Calpains 5, 6, 7, 8b, 10, and 15 are atypical calpains, in that some of their domains have been deleted or replaced. They lack domain IV and therefore presumably do not associate with the 28-KDa subunit.



Calpains. Figure 1 Domain structures of calpain superfamily. Figure kindly provided by Dr. Hiroyuki Sorimachi, Laboratory of Biological Function, University of Tokyo, Japan.

Calpain Regulation

An intricate strategy for the regulation of calpain activity seems necessary since calpain is an abundant cytoplasmic **protease** and large amount of proteins can be identified as substrates. Among them are transcription factors, transmembrane receptors, signaling enzymes, and cytoskeleton proteins. Most of the substrates are cleaved in a limited fashion, resulting in stable protein fragments that can have functions different from those of their intact form. Recognition and proteolysis seems to be controlled by multiple determinants, including the formation of secondary structures and the existence of specific recognition sequences. There is a significant preference for particular sequences immediately surrounding the site of proteolysis. Calpain contains many sites of **phosphorylation** that are physiologically relevant and regulate its activity. Also membrane localization of calpains is an important mechanism for this regulation. It seems that its interaction with the membrane may facilitate the binding to the substrate. Once activated on the membrane, the calpain presumably diffuses into the cytosol and becomes

resistant to the inhibitory action of the calpastatin, the calpain endogenous inhibitor. In the presence of Ca^{2+} , there is a rapid autolysis of calpain. The same autolysis reduces mass of the 80-KDa and 28-KDa subunits of calpain by cutting out some of its amino acids. Ca^{2+} levels, required to initiate autolysis, are as high as or even slightly higher than the levels required for proteolytic activity and are much greater than the free Ca^{2+} levels in living cell. The presence of phospholipids, with phosphatidylinositol, lowers the Ca^{2+} levels required for this autolysis. However, autolysis of calpains does not unblock the active sites as autolysis of other cysteine proteases do. It seems, therefore, that autolysis has some important role in calpain function. The nature of this role is not yet clear. It is possible that the peptides released during autolysis of the calpains may have important properties.

Physiological and Pathological Conditions Associated to Calpains

The calpain system has a number of different roles in cells, including remodeling of cytoskeleton attachments

to the plasma membrane during cell fusion and cell motility, proteolytic modification of molecules in signal transduction pathways, degradation of enzymes controlling progression through the cell cycle, regulation of gene expression, *substrate* degradation in some apoptotic pathways, and an involvement in long-term potentiation. With so much to contribute in the physiological aspects of the cell, it is not surprising that a great number of pathological conditions may also be associated to calpains disturbances (Table 1). They include type II diabetes, cataract, Duchenne's muscular dystrophy, Limb girdle muscular dystrophy (LGMD2A), Parkinson's disease, Alzheimer's disease, rheumatoid arthritis, ischemia, stroke and brain trauma, various platelet syndromes, hypertension, liver dysfunction, and some types of cancer (several oncogenes and tumor suppressor genes products are substrates for members of the calpain family). Abnormalities in expression of calpain homologs, calpain 3a, calpain 9, and calpain 10, are related to LGMD2A, gastric cancer, and type II diabetes, respectively. The others "calpain pathologies" involve loss of Ca^{2+} homeostasis by cell/tissues and inappropriate/excessive degradation of proteins that are known calpain substrates.

Drugs

Calpain inhibition may represent an important mechanism for future drug development. Control of calpain activity may limit the invasive properties of cells and thereby provides a possible mechanism to limit the invasiveness of tumors or inhibits the development of chronic inflammation. For the moment, pharmacological inhibitors of calpains are still not capable of differentiating among different calpain isoforms in cellular systems or in vivo. The importance of calpains in diseases will continue to stimulate the development of new and better inhibitors.

Calpain Inhibitors

More than 50 endogenous and exogenous inhibitors of the calpains have been described as either transition-state reversible or irreversible inhibitors. The first transition-state inhibitors were the peptide aldehydes (e.g., leupeptin). Using this compound, new ones were synthesized that exhibited improved membrane permeability and calpain specificity (e.g., calpeptin). Other groups of inhibitors have since been discovered: α -dicarbonyls (originally developed as serine protease inhibitors), nonpeptide quinolinecarboxamides,

Calpains. Table 1 Examples of pathological conditions that have been associated to the calpains (Adapted from Goll et al.)

Diseases	Observations
LGMD2A	Caused by mutations in the <i>calpain-3</i> gene and probable loss of calpain-3 proteolytic activity ³³
Gastric cancer	Associated to downregulation of <i>Capn9</i> gene ³⁴
Type 2 diabetes mellitus	Mutations in intron 3 of <i>Capn10</i> gene is associated with increased incidence of type 2 diabetes in some populations ³⁵
DMD and BMD	DMD and BMD are caused by the absence or deficiency of dystrophin a membrane-associated protein, resulting in increased Ca^{2+} concentration in muscle, loss of Ca^{2+} homeostasis, and inappropriate calpain activity ³⁶
Alzheimer's disease	Amount of m-calpain in the cytosolic but not the membranous fractions and in the neurofibrillary tangles of brain from Alzheimer's patients is increased ^{37,38}
Cataract formation	Ca^{2+} influx activates m-calpain, the predominant calpain in lens, cleaving α - and β - but not γ -crystallins. The crystallin fragments aggregate to form cataracts ³⁹
Myocardial infarcts	Ca^{2+} homeostasis is lost in ischemic areas, triggering inappropriate calpain activity. Desmin and α -spectrin are degraded in ischemic hearts by synthetic calpain inhibitors. Protein and mRNA levels of m and u-calpain increase after myocardial infarction ^{40–43}
Multiple sclerosis	The 150-kDa calpain specific degradation product of α -spectrin increases 50% in human MS plaques. ⁴⁴ Degradation of the 68-kDa neurofilament protein is inhibited by a synthetic calpain inhibitor ⁴⁵
Obsessive-compulsive disorders	Erythrocytes from patients with obsessive-compulsive disorder have significantly higher calpain activities than normal controls which could not be attributed to differences in memory function ⁴⁶
Neuronal ischemia (stroke)	Calpastatin is degraded by calpain to a membrane-bound 50-kDa polypeptide in ischemic brain. ⁴⁷ Calpains participate in both apoptosis and necrosis in tissue damage in ischemic areas

LGMD, limb girdle muscular dystrophy; DMD, Duchenne muscular dystrophies; BMD, Becker muscular dystrophies

nonpeptide α -mercaptoacrylic acids, and phosphorus derivatives. The acid derivatives are particularly interesting because they mediate their inhibitory activity by interacting with Ca^{2+} -binding domain of the calpain I and II large subunits. Irreversible inhibitors include the epoxysuccinates (nonspecific cysteine protease inhibitors), acetoxymethyl ketones, halomethyl ketones, sulfonium methyl ketones (potent and highly calpain II selective), and diazomethyl ketones. The epoxysuccinate inhibitor E64 is a commonly used irreversible calpain inhibitor. Drugs such as iodoacetate, iodoacetamide, and N-ethylmaleimide, which are inhibitors of cysteine proteases, are also commonly used as inhibitors.

Calpastatin

Calpastatin is the only known ►protein inhibitor specific for the calpains. It is a heat-stable protein, with molecular mass varying from 34 to 300 KDa and resistant to a wide variety of denaturing agents. Although, only one calpastatin gene exists on chromosome 5, the use of different promoters or alternative splicing mechanisms allows the presence of different calpastatin isoforms produced from this single gene. Frequently, more than one isoform exists in a single tissue. Neither calpastatin nor calpastatin-like activities have been reported in invertebrate's tissues, and genes having sequence homologies to calpastatin have not been detected in *Drosophila*, *C. elegans*, or *S. Cerevisiae*. Hence calpastatin may be restricted to vertebrates. The intracellular level of calpastatin correlates directly with calpain activation and the affinity of calpastatin for the activated forms of the calpains is greater than its affinity for the proenzyme, indicating that both structural and conformational changes due to autolysis favor formation of the enzyme–inhibitor complex. Ca^{2+} is required for calpastatin to bind and to inhibit the calpains. It is also known that Ca^{2+} concentrations required to make this inhibition is dependent on the calpain molecule and this inhibition is reversible, releasing the calpain in an undegraded form. The calpain/calpastatin interaction has been well characterized. It is clear that calpastatin has an important role in regulating activity of calpains, but exactly how it acts in this regulation is not well understood. However, it is not difficult to realize that this specific inhibitor has possible therapeutic applications in diseases involving the calpains.

- Non-viral Peptidases
- Ca^{2+} -binding Proteins

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Calsequestrin

Calsequestrin is the major calcium storage protein of the sarcoplasmic reticulum in skeletal and cardiac muscles. It is highly acidic and has a large capacity for Ca^{2+} . Calsequestrin functions to localize calcium near the junctional face of the terminal cisternae from which calcium can be released into the cytosol via the ryanodine receptor.

- Ryanodine Receptor

cAMP

- Cyclic Adenosine Monophosphate

cAMP-GEFs

cAMP-binding Guanine Nucleotide Exchange Factors.

- Small GTPases

Camptothecins

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Definition

Camptothecin is a plant alkaloid that selectively targets topoisomerase I (Top1). It produces DNA damage leading to the destruction of eukaryotic cells. Because

of selectivity against cancer cells, camptothecin derivatives are used as anticancer drugs. Camptothecin comes from *Camptotheca acuminata*, a deciduous tree found in southern China. Stem woods of *Nothopodytes foetida* (previously known as *Mappia foetida*) found in the Western Ghats of India are an even better source of camptothecin.

Historical Perspective

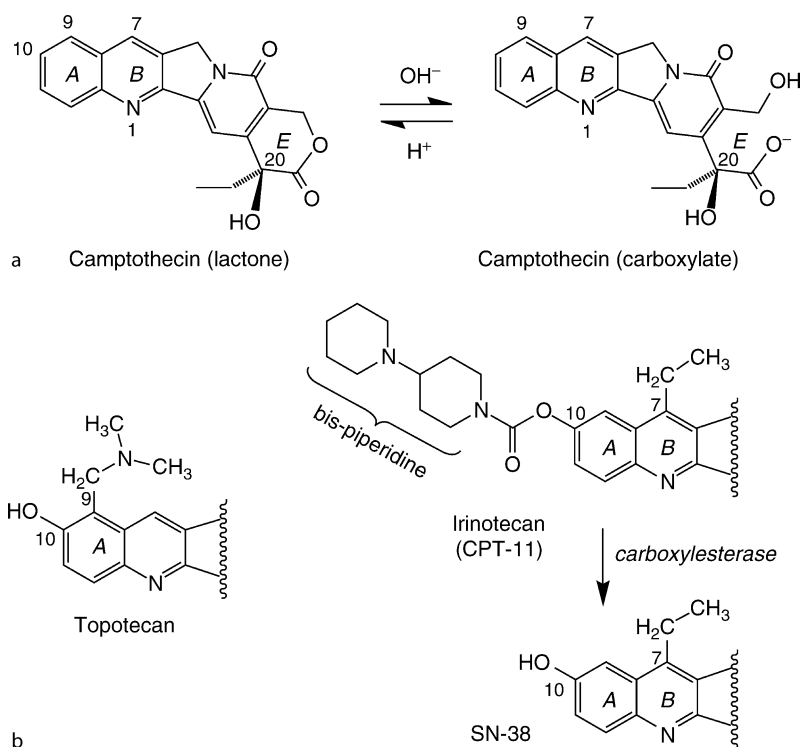
Camptothecin was discovered as an active anticancer drug isolated from the bark of *Camptotheca acuminata*. The anticancer activity of camptothecin was discovered in the 1960s by the National Cancer Institute (NCI) as part of a systematic effort to screen for novel anticancer agents derived from natural products. Monroe Wall and Mansuhk Wani identified the chemical structure of camptothecin. They also identified the chemical structure of taxol, again under the auspices of the NCI. Susan Horwitz was contracted by the NCI to elucidate the anticancer mechanisms of camptothecin. She found in the early 1970s that camptothecin induced DNA breaks and arrested DNA and RNA synthesis. However, it is approximately 12 years later, only after DNA **▶topoisomerase I** (Top1) had been identified in human cells, that Leroy Liu and his coworkers found that Top1 was the cellular target of camptothecin [reviewed in [1]].

Camptothecin is highly insoluble, which facilitated its crystallization and chemical structure determination.

The first clinical trials were performed in the 1970's using a sodium salt derivative with an open E-ring (Fig. 1). However, the clinical efficacy was limited and severe bladder toxicity led to the termination of the clinical trials. The poor efficacy of the camptothecin sodium salt in those clinical trials was probably due to the fact that the open E-ring form of camptothecin (carboxylate derivative) is inactive as a Top1 inhibitor. Following the identification of Top1 as a target of camptothecin, water-soluble derivatives were produced by the pharmaceutical industry. Two of these water-soluble derivatives have been approved by the FDA for cancer treatment in the early 2000s: **▶topotecan** and **▶irinotecan**.

Mechanism of Action

The natural isomer of camptothecin: 20-*S*-camptothecin (Fig. 1) is a highly specific Top1 inhibitor. By contrast, the 20-*R* derivative obtained by chemical synthesis by Monroe Wall and Mansuhk Wani is inactive against Top1 and as an antitumor agent. Top1 is the only cellular target of camptothecins as human cell lines selected for drug resistance consistently mutate their Top1 in such a way that those mutant enzymes become highly resistant to camptothecins while remaining catalytically active. TOP1 is an essential gene in metazoans whereas yeast strains with TOP1 gene deletion are viable but immune to camptothecins.



Camptothecins. Figure 1 Chemical structures.

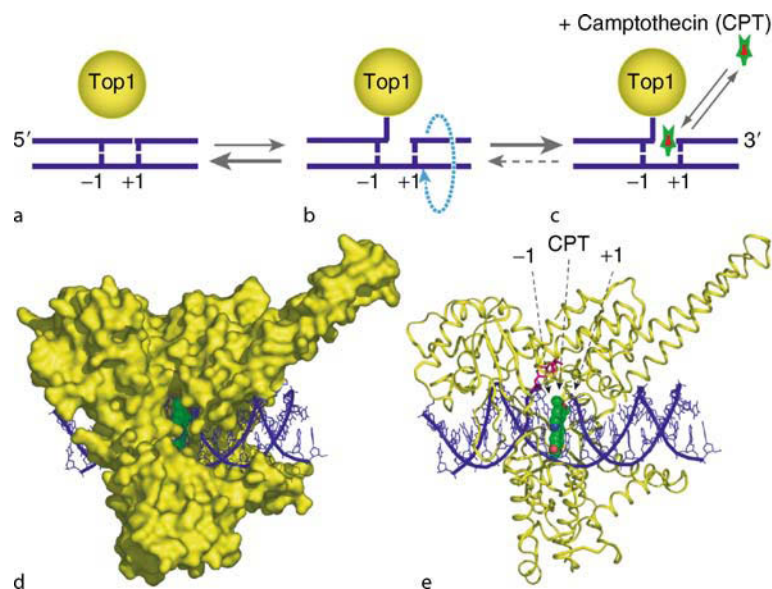
Top1 is an essential enzyme in metazoans because it is required to relax DNA supercoiling generated by chromatin remodeling during transcription and replication (Figs 2a, b). Camptothecin binds to the Top1-DNA cleavage complex by intercalating between the base pairs flanking the DNA cleavage site (Fig. 2c). As a result, camptothecins slow down the re-ligation of Top1 cleavage complexes. Cocrystal structures show that camptothecins not only binds to the Top1-DNA complex by stacking interactions with the DNA but also by forming a network of hydrogen bonds with amino acids residues of Top1. Those amino acid residues are those whose mutations confer CPT resistance (Fig. 2d, e). Thus, CPT is a paradigm for “▶interfacial inhibitors”, i.e. drugs that bind at the interface of macromolecular complexes [2].

Cancer cells are killed by camptothecins as a result of the trapping of Top1-DNA cleavage complexes. Those

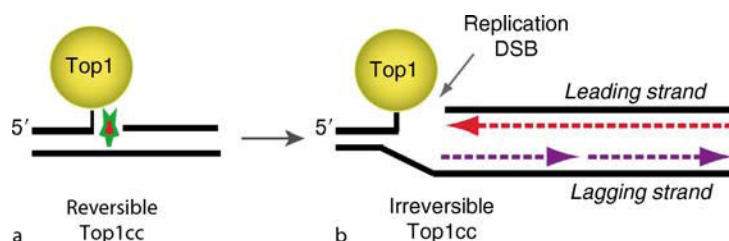
trapped Top1 cleavage complexes preferentially kill replicating cells as they produce collision with DNA replication forks (Fig. 3).

Clinical Use (Including Side Effects)

Topotecan (Hycamtin®) is routinely used to treat ovarian cancers and small-cell lung cancers (SCLC). It is given by intravenous infusion. Hematological toxicity is a common side effect due to the destruction of bone marrow progenitors. Infections are due to loss of white blood cells, bruising or bleeding to the loss of platelets, and anemia with fatigue to loss of red blood cells. Within a day following infusion, patients generally feel sick with nausea and possibly vomiting. They can be controlled with anti-emetic drugs. Loss of appetite for a day or so is common. Patients may also feel tired during the first weeks of treatment. Hair loss starts 3–4 weeks after the first dose. It is temporary. Hair regrows once the



Camptothecins. Figure 2 (a) Topoisomerase I (Top1) is an abundant enzyme. It is mostly associated noncovalently with chromatin. (b) Top1 relaxes DNA by making single-strand breaks that are generated by the covalent linkage of Top1 to the 3'-end of DNA. (c) Camptothecin binds reversibly to the Top1-DNA cleavage complex and slows down DNA re-ligation. (d) Ternary complex including Top1 (yellow), DNA (dark blue ribbons), and camptothecin (green and red in the middle). (e) Same structure except Top1 is in ribbon representation.



Camptothecins. Figure 3 Conversion of reversible Top1-DNA cleavage complexes into DNA damage by collision of a replication fork (the DNA polymerase complex is not shown).

treatment is finished. Because of potential ►**teratogenic** effects, it is recommended to use contraception during topotecan treatment and a few months afterwards.

Irinotecan (CPT-11) is approved for colorectal tumors. It is given by intravenous infusion. The most severe side effect is diarrhea, which can be severe and needs to be treated by a physician. Temporary liver dysfunction is generally asymptomatic. The other side effects are the same as those produced by topotecan.

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Cancer, Molecular Mechanisms of Therapy

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Synonyms

Malignant tumor; Malignant neoplasm

Definition

Cancers are tumors that have acquired the ability to invade and disrupt surrounding normal tissues. Such lesions can arise in any tissue of the human body, and so cancers represent a heterogeneous group of diseases that have certain biological features in common.

A tumor begins as an outgrowth of a clonal population of cells that follows a loss of tissue homeostasis, and progresses into a cancer as it grows in size and becomes invasive. During the later stages of cancer progression, cancer cells can enter the bloodstream or lymphatic system and grow in distant sites. This process is known as metastasis. The invasive and metastatic properties of cancers lead to the lethal destruction of normal tissues.

Basic Mechanisms

Many anticancer agents currently in use, including chemotherapeutic drugs and radiation, are potent inducers of ►**apoptosis** and ►**cell-cycle arrest**. It is believed that induction of these molecular pathways is central to the efficacy of such agents [1]. Genetic and epigenetic alterations that contribute to tumorigenesis

often disable the pathways that lead to apoptosis and growth arrest and therefore affect the way that cancer cells respond to therapy. Recent advances in the understanding of how cellular pathways of growth and death work have led to insights as to how therapy might be optimized.

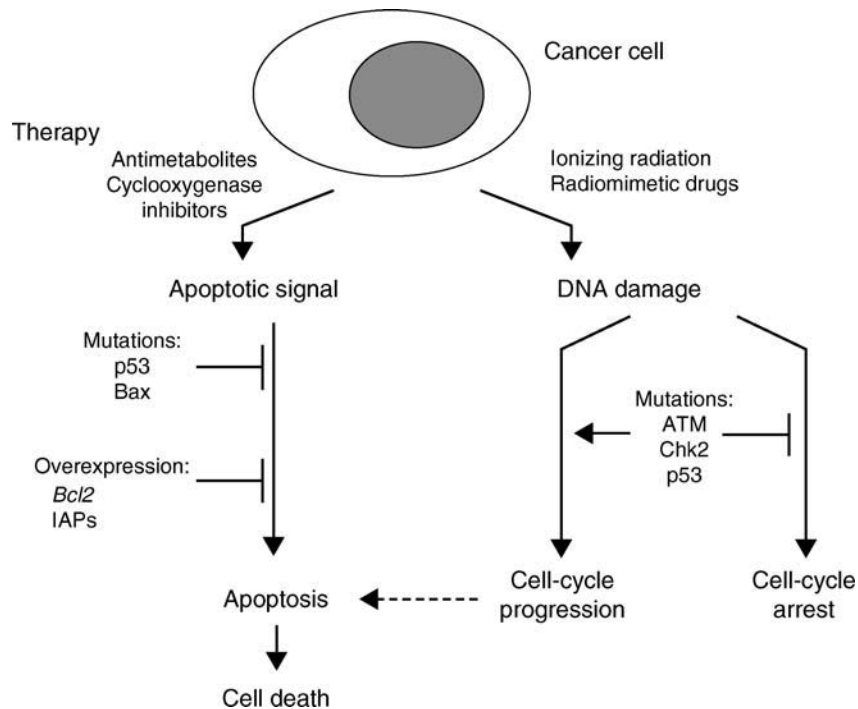
The regulatory apparatus that controls the cell division cycle is the frequent target of inactivating mutations, and some of these mutated genes can be inherited in cancer-prone families. Similarly, mutations can affect apoptotic pathways. Many cancer-derived cell lines demonstrate a resistance to experimental apoptotic stimuli and recent evidence supports the idea that alterations in genetically defined apoptotic pathways are actively selected for during tumor development. It thus appears that inactivation of the pathways that lead to cell-cycle arrest and apoptosis confer a selective growth advantage.

The relative contributions of cell-cycle arrest and apoptosis to the death of tumor cells following therapy remain a point of controversy [2]. In vitro, cell death triggered by anticancer agents can occur as a result of an immediate apoptotic program or, alternatively, following a substantial delay as a result of dysregulated cell-cycle arrest. These pathways and the drugs that trigger them have been experimentally defined in a colon cancer cell system (Fig. 1). At present it appears likely that different pathways dominate in different cell types. Furthermore, it is important to understand that the experimental systems currently in use are imperfect representations of tumors and these no doubt fail to fully represent complex cellular responses of human cancers. The development of model systems that better emulate cancers in situ will likely aid in understanding how death pathways can work in a clinical setting.

Apoptosis

A substantial amount of indirect evidence supports the contention that the induction of apoptosis in tumor cells is critical to successful therapy. Cancer therapy might therefore be viewed as an attempt to induce apoptosis in a population of cells that have undergone selection for apoptotic defects. If correct, this hypothesis would suggest why cancer therapy is in many cases unsuccessful. However, recent studies indicate that this fundamental problem can be circumvented. Progress in the identification of molecules key to the cell death pathways has led to a growing understanding of how apoptosis occurs [3]. It has become clear that pathways to apoptosis are numerous and often interconnected. A solution to the clinical problem of therapeutic resistance, then, may lie in the fact that there appears to be multiple ways that a cell death program can be implemented.

Apoptosis occurs as a result of a cascade of proteolysis that culminates in the destruction of the cell. Apoptotic proteolysis is catalyzed by the ►**caspase** family of



Cancer, Molecular Mechanisms of Therapy. Figure 1 Cancer therapy and cell death. There are at least two pathways by which therapeutic and preventive agents can cause the death of colon cancer cells. A. Antimetabolites and cyclooxygenase inhibitors can trigger apoptotic signals. The apoptotic signaling pathways can be disrupted in different stages by a number of cancer-related gene mutations and overexpressed proteins, leading to relative therapeutic resistance. B. Therapeutic agents that damage chromosomal DNA can activate a signal transduction pathway that normally results in a protective arrest of the cell-cycle. When disrupted by cancer-related mutations, this pathway can fail resulting in progression of the cell-cycle, which leads to the dysregulated replication and segregation of damaged chromosomes. Cells that escape cell-cycle arrest are subsequently eliminated by apoptotic effector proteins, resulting in relative therapeutic sensitivity.

proteases and can occur via the activation of one of two major pathways. In the intrinsic, or mitochondrial apoptotic pathway, an intracellular death signal causes the disruption of mitochondria, which liberates into the cytoplasm proteins that facilitate caspase activation. Proapoptotic members of the *Bcl2* family appear to play a central role in the physical disruption of the mitochondrial membranes. An extrinsic apoptotic pathway uses the same downstream effector proteases as the intrinsic pathway, but in this case the death signal originates from the cell surface. External death-inducing ligands bind a family of death receptors such as CD95 (also known as Fas and Apo-1), tumor necrosis factor receptor 1 and TRAIL (also known as DR5 and KILLER). Ligand binding induces multimerization of these receptors that results in caspase activation.

Negative regulation of apoptosis is effected by inhibitor of apoptosis proteins. Another means of controlling apoptosis is by altering the stabilization of mitochondria, which is largely determined by the interplay between the pro- and antiapoptotic members of the *Bcl2* family [4].

Alterations to the *P53* gene are the most common genetic defects known in cancer [5]. The protein product of *P53* is involved in a number of pathways that directly and indirectly lead to apoptosis. Many genes that are involved in apoptosis can be induced by this protein, which is a transcriptional transactivator. The emerging hypothesis is that p53 is a central node of a complex apoptotic network that may function differently in diverse cell types and tissues. For example, *Bax*, the prototype proapoptotic member of the *Bcl2* family, can be transcriptionally induced by p53 in certain, but not all, cell types. Like *p53*, *Bax* can modulate the extent to which cells are sensitive to apoptosis caused by therapeutic agents.

Cell-Cycle Regulation

Ionizing radiation and radiomimetic drugs that cause DNA damage are among the most frequently used anticancer agents. In the presence of damaged chromosomes, cellular pathways are triggered which bring to the progression of the cell-cycle to a halt, a status known as cell-cycle arrest. Cell-cycle arrest is mediated

by the activation of checkpoints. Checkpoints block specific processes that occur during cell growth. In this way, cells with intact checkpoints prevent the replication and segregation of damaged chromosomes. In cells that have disrupted checkpoint pathways, DNA damage can lead to the aberrant progression of the cell-cycle, which can result in lethal reduplication of genomic DNA and inappropriate timing of cell division. Analysis of genetic alterations that occur in hereditary cancers suggests that the G₂ checkpoint, which prevents the entry of cells with damaged chromosomes into mitosis, is a particularly important target of mutational inactivation during carcinogenesis.

In addition to its role as a mediator of apoptosis, the p53 protein is part of a checkpoint mechanism that causes arrest of the cell-cycle. Whether p53 activation causes apoptosis or cell-cycle arrest depends upon the stimulus applied and upon the cell type being observed [5]. In response to DNA damage, p53 can be activated by the Chk2 kinase, which is in turn activated by the ATM (Ataxia Telangiectasia Mutated) kinase. The checkpoint functions of these tumor suppressor genes, which are mutated in a broad spectrum of sporadic and inherited cancers, supports the idea that the inactivation of the G₂ checkpoint is a frequent event during carcinogenesis and that many tumors are likely to be checkpoint defective. An understanding of how cancer cells differ from normal cells in their responses to DNA damage appears likely to prove useful in devising therapy of maximal efficacy.

Pharmacological Intervention

Most of the therapeutic anticancer agents currently in use were developed empirically, without any prior knowledge of apoptotic or cell-cycle control mechanisms. Subsequently, it has become apparent that apoptosis and cell-cycle arrest are triggered upon exposure of tumor cells to these agents. Recent advances in our understanding of the molecular mechanisms of apoptosis and cell-cycle regulation have illuminated the mode of action of many of the common anticancer agents and suggested why some cancers are resistant to therapy.

Therapeutic Induction of Apoptosis

Many of the agents currently in use for the therapy of cancer can trigger apoptosis in cancer cells. Cancer-associated alterations of the genes that regulate apoptosis would therefore be predicted to affect sensitivity to these agents.

One commonly used agent is the ▶**antimetabolite** 5-fluorouracil (5-FU), which is frequently used as an adjuvant therapy in conjunction with surgical excision in the treatment of solid tumors. p53 can directly trigger apoptosis in cells exposed to 5-FU in vitro [1]. In addition, there is a substantial amount of clinical

evidence that suggests that p53 status may be predictive of the response of patients to 5-FU therapy.

The pro- and antiapoptotic members of the *Bcl2* family affect cellular sensitivity to apoptosis and thus to many chemotherapeutic agents [4]. For example, overexpression of the antiapoptotic genes *Bcl2* and *Bcl-XL* in some cell lines and tumors can confer resistance to apoptosis triggered by ionizing radiation. Conversely, overexpression of proapoptotic *Bax* in experimental tumors can induce apoptosis directly or render such tumors more sensitive to cisplatin and 5-FU.

Nonsteroidal antiinflammatory drugs, such as sulindac and indomethacin, are important chemopreventive agents for patients genetically predisposed to colorectal cancer. This class of compounds, which bind and inhibit ▶**cyclooxygenases**, has been found to cause apoptosis in cultured gastrointestinal epithelial cells. Strikingly, the experimental deletion of the *Bax* gene in tumor-derived colon cancers has been found to disrupt the apoptotic response to this class of drugs, demonstrating the potential importance of this pathway in chemoprevention.

Therapeutic Activation of Cell-Cycle Checkpoints

While induction of apoptosis is the most well recognized molecular mechanism of action of anticancer agents, it is becoming clear that many agents can exploit the checkpoint defects that often occur during cancer development. In fact, ionizing radiation and ▶**radiomimetic** drugs such as doxorubicin and bleomycin that cause DNA damage are among the most frequently used anticancer agents.

Exposure of many types of normal cells to DNA damaging agents results in the activation of cell-cycle checkpoints, which implement a protective cell-cycle arrest. In cancer cells that have checkpoint defects, such treatment would be predicted to lead to arrest failure, cell-cycle dysregulation, and eventual cell death. The observed sensitivity of cancer cells, then, is thought to result from their failure to undergo a protective cell-cycle arrest in response to treatment. Ultimately, the cell death that follows cell-cycle dysregulation may occur through apoptotic pathways, demonstrating a complex relationship between these fundamental processes.

Though DNA damage-based therapies have been in use for many years, it has remained unclear why such treatment often causes the selective death of tumor cells while sparing adjacent normal tissue. The genetic alterations that occur in cancers that alter the DNA damage response may explain why such therapy can be efficacious.

- ▶ **Antineoplastic Agents**
- ▶ **Antimetabolids**
- ▶ **Alkylating Agents**

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allowed in most countries. THC is medically used as antiemetic in patients receiving cancer chemotherapy and administered to AIDS patients to stimulate appetite. THC binds to and activates ►cannabinoid receptors (CB₁ and CB₂) belonging to the superfamily of ►G-protein coupled receptors.

- Appetite Control
- Antiobesity Drugs
- Emesis
- Endocannabinoids

Cannabinoid Receptors

The two G-protein-coupled receptors for Cannabis psychotropic component, Δ^9 -tetrahydrocannabinol (THC) cloned to date, the cannabinoid CB₁ and CB₂ receptors.

- Endocannabinoids

Cannabinoids

Group of compounds which naturally occur in the hemp plant, *Cannabis sativa*. Most of them are insoluble in water. The most abundant cannabinoids are Δ^9 -tetrahydrocannabinol (THC), its precursor cannabidiol and cannabinol, which is formed spontaneously from THC. Cannabinoids exert their effects through G-protein coupled cannabinoid receptors (CB₁/CB₂).

- Endocannabinoids

Cannabis

The plant *Cannabis sativa* produces the psychoactive drug Δ^9 -tetrahydrocannabinol (THC). Recreationally, THC is consumed in form of marijuana (dried flowers and leaves) or hashish (resin). The cultivation and possession of *Cannabis* for recreational use is not

Capsaicin

Capsaicin, also known as N-Vanillyl-8-methyl-6-(E)-noneamide, is the most pungent of the group of compounds called capsaicinoids: It is a common ingredient in varieties of pepper such as habanero, Thai, tabasco, cayenne etc. One target with which capsaicin interacts is the capsaicin receptor, an ion channel belonging to the superfamily of TRP channels. Because of the structural relation to other TRP channels and because the vanilloid moiety is an essential component of capsaicin, the capsaicin receptor is also called TRPV1 or vanilloid receptor (VR1). It is involved in heat and pain perception.

- Nociception
- Non-selective Cation Channels
- TRP Channels

Capsaicin-sensitive Primary Afferent Neurons

These are a subset of sensory neurons having their cell bodies (small to medium size) in dorsal root and in cranial nerve ganglia and possessing nonmyelinated (C-type) or thinly myelinated (A-delta type) fibres. This subset of neurons express transient receptor potential vanilloid type 1 (TRPV1, or vanilloid, or capsaicin receptor) that is excited by capsaicin, the pungent ingredient of chilli. These neurons have been classified as polymodal nociceptors because they can be excited by various noxious stimuli.

- Capsaicin
- Tachykinins and their Receptors
- TRP Channels

Capsid

A capsid is a proteinaceous shell encasing the viral genome. Viral capsids are polymeric, ordered structures composed of one or more virus encoded subunits.

► Antiviral Drugs

CaR

► Ca^{2+} -sensing Receptor

Carbon Monoxide

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Synonyms

Carbonic oxide; Coal gas; CO

Definition

Carbon monoxide (CO) is a colorless and odorless gas molecule. This inorganic compound, at standard temperature and pressure, is chemically stable with low solubility in water but high solubility in alcohol and benzene. Incomplete oxidation of carbon in combustion is the major source of environmental production of CO. When it burns, CO yields a violet flame. The specific gravity of CO is 0.96716 with a boiling point of -190°C and a solidification point of -207°C . The specific volume of CO is 13.8 cu ft/lb (70°F).

Basic Characteristics

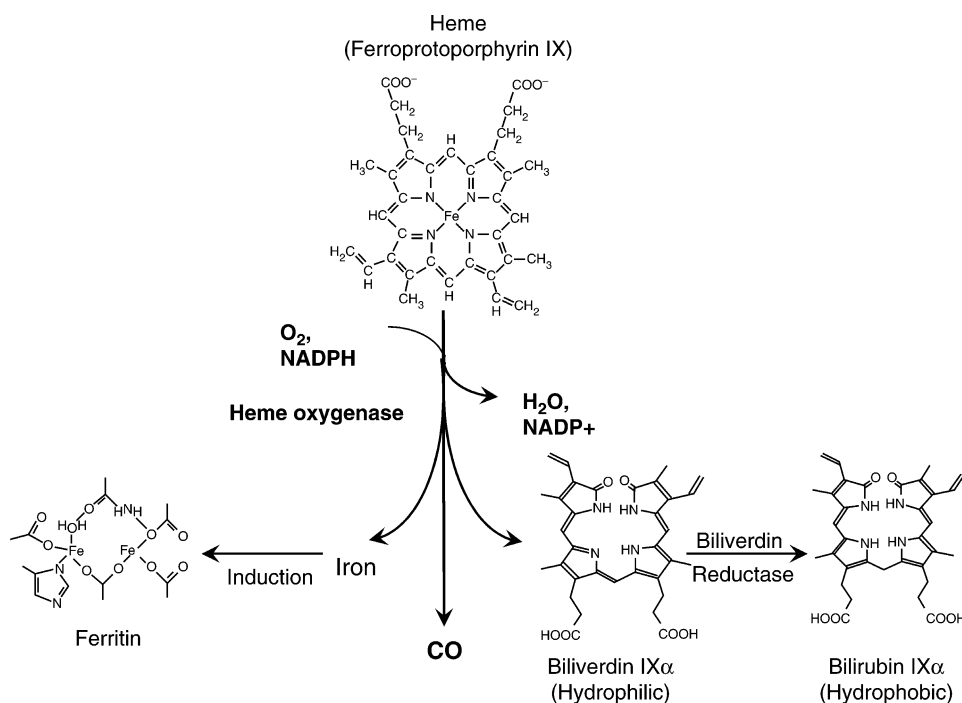
All mammalian cells are virtually capable of producing CO with heme as the main substrate (Fig. 1) [5]. Enzymatic heme metabolism *in vivo* is mainly catalyzed by heme oxygenase (HO). In the presence of HO, the porphyrin ring of heme is broken and oxidized at the α -methene bridge, producing equimolar amounts of CO, ferrous iron, and biliverdin. Three isoforms of HO have been identified. Inducible HO-1 (32 kDa) is mostly recognized for its upregulation in response

to physiological and pathophysiological stimulations to increase oxidation of heme and production of CO and biliverdin. HO-2 (36 kDa) is a constitutive form of HO with relative stable expression level in almost every type of mammalian cells. HO-3 is also constitutively expressed in a limited number of mammalian cells. The function of HO-2 appears to be the regulation of basal heme metabolism. As a poor heme catalyst, the biological function or necessity of HO-3 is not clear.

CO has been known as a silent or invisible killer because severe CO intoxication leads to death, due to its binding to hemoglobin and reducing the oxygen carrying capacity of blood. Neurological disorders are often encountered in patients with mild CO intoxication, including impaired vision and coordination, headaches, dizziness, faintness, disorientation, nausea, and fatigue, etc.

At physiologically relevant concentrations, CO plays profound and important physiological roles in regulating cellular functions. In the cardiovascular system, CO exerts different vasorelaxant effects on different types of blood vessels, protects myocardial cells and isolated heart preparations from ischemia/reperfusion damage, and inhibits platelet aggregation [2]. Modulation of hypothalamic-pituitary-adrenal axis, control of circadian rhythm, and adaptation of odor response are examples of the neuronal effects of CO. Moreover, endogenous CO is involved in nociception and chemoreception, thermal regulation, vision, hearing, learning, memory, and behavior. Due to its strong anti-inflammatory and antiproliferation effect, CO may also suppress the development of pulmonary fibrosis. A basal level of endogenous CO is important in supporting female reproductive processes and lactation, and in regulating penile erection and ejaculation in male. Depending on the concentration range, CO affects baseline bile output and the occurrence of choleresis. Increased renal production of CO has been shown to be renal protective. By modulating calcium handling of pancreatic islet beta cells, CO increases insulin release. Abnormalities in endogenous CO metabolism contribute to various pathological situations, including hypertension, neurodegeneration disorders, cardiac failure, and inflammation, to name a few [5].

The cellular and molecular mechanisms for the biological functions of CO are closely related to the chemical nature of CO. As a ►**gasotransmitter**, CO is freely permeable to cell membrane and its cellular effect is not mediated by specific membrane ►**receptors**. CO binds to reduced iron [Fe(II)] in the center of heme-containing proteins. This binding subsequently alters functions of the affected heme-containing proteins, such as hemoglobin, myoglobin, prostaglandin endoperoxide synthase, NOS, catalase, peroxidases, respiratory burst oxidase, pyrrolases, cytochrome c oxidase, cytochrome P450, soluble guanylyl cyclase



Carbon Monoxide. Figure 1 Heme oxygenase catalyzed heme metabolism (from Pharmacol Rev 57:585–630,

(sGC), tryptophan dioxygenase, and many others. The binding of CO to heme-containing proteins can lead to either activation or inactivation of these proteins. The binding of CO to HbA inhibits its O_2 carrier function since both O_2 binding to, and its release from HbA are suppressed. The binding of CO to sGC, on the other hand, activates this enzyme. The activation of sGC leads to increased production of cGMP , which activates cGMP-dependent protein kinase. The latter phosphorylates several important regulators of $[Ca^{2+}]_i$, inhibiting Ca^{2+} gates and activating Ca^{2+} pumps on the endoplasmic reticulum, and activating K_{Ca} channels on the plasma membrane. cGMP also regulates several classes of phosphodiesterases and is itself rapidly degraded by phosphodiesterases.

In addition to intracellular heme-containing proteins, big-conductance calcium-dependent K^+ (BK_{Ca}) channels and calcium-spark activated transient K_{Ca} channels in plasma membrane are also targeted by CO [3]. As well known, nitric oxide (NO) also activates BK_{Ca} channels in vascular smooth muscle cells. While both NO and CO open BK_{Ca} channels, CO mainly acts on alpha subunit of BK_{Ca} channels and NO mainly acts on beta subunit of BK_{Ca} channels in vascular smooth muscle cells. Rather than a redundant machinery, CO and NO provide a coordinated regulation of BK_{Ca} channel function by acting on different subunits of the same protein complex. Furthermore, pretreatment of vascular smooth muscle

cells with NO abolished the effect of subsequently applied CO on BK_{Ca} channels. This can be explained by an altered interaction of beta subunit and alpha subunit of BK_{Ca} channels induced by NO, which prevents the interaction of CO with beta subunit of BK_{Ca} channels. It has been shown that in peripheral resistance and cerebral arterioles, activation of K_{Ca} channels by CO is the major molecular mechanism for CO-induced vasorelaxation. In other types of blood vessels, effect of CO on K^+ channels may become less important in comparison with activation of the cGMP pathway by CO [2].

Drugs

Therapeutic Application of CO

To prepare CO solution for the experimental purpose, it is recommended to bubble 20 ml of stock solution in a sealed glass tube with a stream of pure CO gas. The bubbling process lasts for 20 min under the pressure of 100 kPa at 37°C [3]. One microliter of this CO-saturated solution is estimated to contain 30 ng of the gas based on the solubility of CO at 37°C , the extent of dilution of the CO-saturated solution, and the assumption that the loss of the added CO from the bath solution at the time of experiments is negligible. The stock solution of CO should be freshly prepared before each experiment and then should be diluted immediately to the desired concentration with the bath solution or culture media.

Approaches to Increase CO Levels

Increasing endogenous CO production or directly applying exogenous CO has not been tested on humans. Ample experimental data, however, have been obtained in different animal species and from various types of cultured cells, supporting the idea of using CO as an effective therapeutic agent.

Increased Endogenous CO Production

As CO is the enzymatic product of HO, upregulation of HO expression would increase endogenous CO level. Human *HO-1* gene has been transferred to experimental rats using retroviral vectors to attenuate the development of hypertension, promote somatic growth, and reduce left ventricular myocardial infarction induced by ischemia/reperfusion myocardial injury. Human *HO-1* gene transfer to rat heart also decreases myocardial lipid peroxidation and the abundance of proapoptotic Bax and proinflammatory interleukin-1 β and increase anti-apoptotic Bcl-2 protein levels. Human *HO-1* gene transfer can be potentially applied to and targeted on patients who have history of cardiac attack episodes or at risk of developing coronary ischemic events. The major concern for *HO* gene transfer resides on the issue of safety of vectors. Nonpathogenic, weakly immunogenic, minimal immune and inflammatory reactions by the host are prerequisites for the application of viral or nonviral factors to humans. Furthermore, success in *HO* gene transfer in experimental animals by no means warrants its success on human beings.

HO-1 expression can also be upregulated by various pharmaceutical drugs or compounds. SnCl₂ and CoCl₂ are among HO-1 inducers that were tested in early time. Applications of these compounds have been discontinued due to their excessive toxicity and high index of animal mortality. Heme is also called reduced hematin. The Fe³⁺ oxidation product of heme is termed hemin. Hemin (Fe³⁺) is reduced to heme (Fe²⁺) in vivo prior to oxidation by HO. Hemin is also called hematin chloride. The elevated heme level itself presents a prooxidant threat. The cell copes with this threat by upregulating HO-1 expression. The latter degrades heme to increase the production of CO. Heme derivatives, such as hemin and heme-L-lysinate, are now of choice for pharmacological induction of HO-1 expression. Blood pressure lowering effect of hemin injection to young spontaneously hypertensive rats (SHR) was observed in late 90s'. Short-period administration of hemin (i.p., 15 mg/kg/day for 4 days) to young SHR significantly upregulated HO-1 expression and lowered blood pressure of these hypertensive animals. Application of heme-L-lysinate (45 μ mol/kg i.p.) or heme-L-arginate (45 μ mol/kg i.p.) to SHR, phenylephrine-induced, and DOCA-salt hypertensive rats has been shown to lower blood pressure within 1 h of the bolus

injection. Among the pitfalls for using HO-1 inducers is potential oxidative damage as both the inducers, such as heme, and some of the products of HO, such as iron, are prooxidants. Therefore, dosage and duration as well as administration route of HO-1 inducers should be carefully designed.

Administration of Exogenous CO

In recent years, CO inhalation at 500–1,000 ppm has been tested as a novel therapeutic approach for certain diseases. In one study, myocardial ischemia/reperfusion damage was induced to rats by occluding the left anterior descending coronary artery for 30 min. Inhalation of 1,000 ppm CO for 24 h significantly reduced the ratio of infarct areas to risk areas and suppressed the migration of macrophages and monocytes into infarct areas, and the expression of TNF- α in the heart. In another study, exposing rats for 20 h/day to CO (200 ppm) for 14 consecutive days induced a 21% increase in right ventricular hypertrophy and a 7% increase in left ventricular hypertrophy. It appears that CO inhalation as a therapeutic avenue should only be given for a short period.

CO-releasing compounds have been produced from certain transitional metal carbonyls. The most recently developed tricarbonylchloro(glycinato) ruthenium(II) (CORM-3) slowly liberates CO in vitro, ex vivo, and in vivo. In addition to its advantageous water solubility, CORM-3 also releases CO in a controllable rate at physiological pH. Many laboratories have tested CORM-3 and highly appreciated its usefulness. For example, CORM-3 administration significantly lowered mean blood pressure in vivo, and relaxed pre-contracted aortic rings in vitro [1].

The oxidation of dichloromethane (DCM), also named methylene chloride, yields CO and CO₂ in vivo, constituting a CO prodrug. Six hours after administration of a single oral dose of DCM (6.2 μ mol/kg) to rats led to an elevated mean maximum COHb level of 9.3%. DCM can also be administered by injection (3 μ mol/kg i.p.) or inhalation (100–500 ppm). The quantity of CO produced per mole inhaled DCM in rats was about 0.7–0.8 mol. The half-life of DCM in vivo is about 3 h and CO released from DCM has a half-life of about 13 h. Oral application of DCM immediately after allogeneic aorta transplantation in rats (500 mg/kg/day for 15–30 days) significantly reduced neointima formation of the vessel wall. Prolongation of liver allograft has also been observed with the application of DCM with diminished apoptosis and preservation of hepatic architecture and function. In another study, an oral dose of 6.2 μ mol/kg DCM in corn oil was given 6 h before induction of liver damage. This treatment protected mice from liver damage based on the measurement of plasma enzyme activities of alanine aminotransferase and caspase-3 activation in liver tissues.

Approaches to Decrease CO Levels

Overproduction of CO *in vivo* is accompanied by overloading of iron and accumulation of bilirubin. The former leads to iron intoxication and the latter kernicterus in neonates. Overproduced CO alone stimulates free radical production in mitochondria and becomes poisonous to selective heme proteins. For these considerations, inhibition of endogenous CO production under specific pathological conditions is demanded. Different pharmacological blockers have been tested for their capabilities to selectively inhibit HO activity and the consequent CO production. The most widely studied and used HO blockers are ► **metalloporphyrins**, including chromium mesoporphyrin (CrMP), manganese protoporphyrin, manganese mesoporphyrin, zinc protoporphyrin (ZnPP), tin mesoporphyrin, and tin protoporphyrin (SnPP) [4]. The acute and chronic applications of metalloporphyrins have been reported. For example, daily injection of ZnPP (45 $\mu\text{mol/kg}$ i.p.) or CrMP (4 $\mu\text{mol/kg}$ i.p.) for 4 days resulted in a striking increase in blood pressure in prehypertensive (4-week-old) and young (8-week-old) SHR. By carefully choosing the concentrations, these metalloporphyrins can specifically inhibit HO without interacting with other cellular components. This is specifically relevant to the reported inhibition of NO synthase and sGC activities by metalloporphyrins. The permeability of the blood–brain barrier to different metalloporphyrins varies. SnPP can easily pass the blood–brain barrier, while ZnPP cannot. This property is important in determining administration routes for applying metalloporphyrin *in vivo* to different organs and tissues.

In addition to pharmacological approach, HO expression can also be downregulated by HO antisense treatment. Once the antisense is tagged to a specific cell type, cell type-specific local production of CO can be inhibited, which is more advantageous than general inhibition of HO activity by pharmacological compounds.

► Nitric Oxide

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Carbon Oxide

► Carbon Monoxide

Carbonic Anhydrase

Carbonic anhydrase (CA) is a zinc-containing enzyme that facilitates the interconversion of CO_2 and HCO_3^- . More than ten carbonic anhydrase isozymes have been identified, some of which are cytoplasmic, such as CA I and CA II, some are membrane bound, such as CA IV, and some are mitochondrial, such as CA V. CA VI is secreted into saliva. CAs have a wide distribution and they participate in all physiological processes that deal with CO_2 and HCO_3^- handling such as cellular pH regulation and acid and ion transport. Highest expression levels are found in red cells (CA I and CA II), but CA activity is also present in lung, endothelial cells, muscle, kidney, ciliary body and lens, pancreatic ducts, salivary gland acini, choroid plexus, osteoclasts, and in other tissues and cells. The observation that sulfanilamide inhibited renal HCO_3^- absorption by inhibition of CA led to the development of the sulfonamide class of diuretics that includes thiazides and loop diuretics. CA II deficiency syndrome is an autosomal recessive disorder characterized by osteopetrosis, renal tubular acidosis, and cerebral calcification and mental retardation while CA VI deficient patients show loss or impairment of taste and smell sensations.

► Diuretics

Carboxypeptidase

An exopeptidase that sequentially releases an amino from the C-terminus of a protein or peptide. Carboxypeptidases are classified in Enzyme Nomenclature according to catalytic type and are included in subclasses 3.4.16–3.4.18.

► Non-viral Peptidases

Carcinogenesis

►Cancer, Molecular Mechanisms of Therapy

Cardiac Glycosides

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Synonyms

Digitalis

Definition

Cardiac glycosides (CG) are potent and highly specific inhibitors of the intrinsic plasma membrane Na^+/K^+ -ATPase, also known as the sodium pump. They modulate electrophysiological properties of the heart and its contractile functions.

Mechanism of Action

The molecular target of cardiac glycosides is the Na^+/K^+ -ATPase (EC 3.1.6.37), which maintains the high sodium and potassium gradients across the plasma membrane, coupled to the hydrolysis of the high-energy phosphate ATP. The gradient is required for the regulation

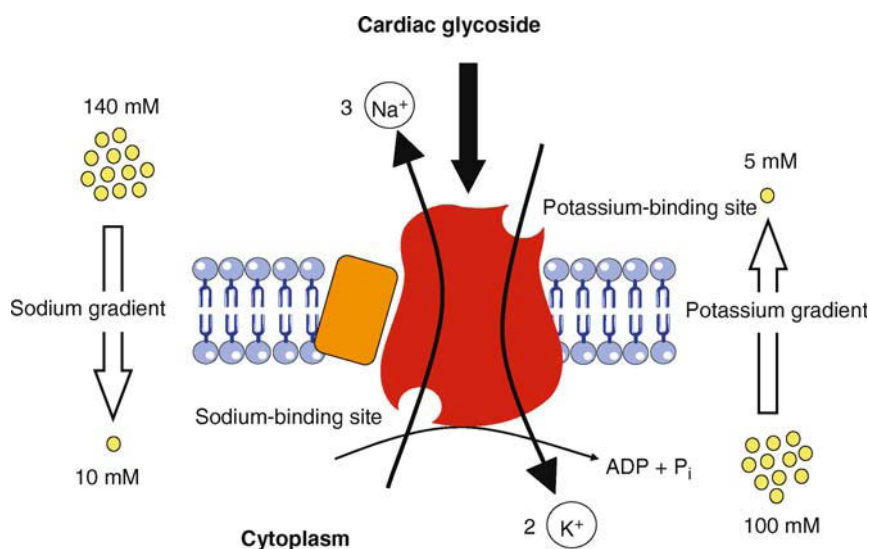
of cell volume, active transport of molecules or the creation and propagation of the action or resting potential of electrically excitable cells. Such are cardiac cells, with a high density of Na^+/K^+ -ATPase in the ►sarcolemma. While potassium is pumped into the cytoplasm, sodium is transported into the extracellular space in a stoichiometric ratio of $3\text{Na}^+:2\text{K}^+:1\text{ATP}$ (Fig. 1).

Inhibition of the Na^+/K^+ -ATPase leads to a loss of potassium and an increase of sodium within the cell. Secondary intracellular calcium is increased via the $\text{Na}^+/\text{Ca}^{++}$ -exchanger. This results in a positive inotropic effect in the myocardium, with an increase of peak force and a decrease in time to peak tension. Besides this, cardiac glycosides increase vagal activity by effects on the central vagal nuclei, the nodose ganglion and increase in sensitivity of the sinus node to acetylcholine.

Molecular Structure of Cardiac Glycosides

Cardiac glycosides are found in several plants and certain toads. Common to these substances is an aglycone-portion (genine) with a steroid structure, a 17β -unsaturated lactone ring, a glycone-portion (sugar) in 3β -position and an OH-group in position 14 (Fig. 2). Glycosides with a 5-numbered lactone ring are classified as cardenolides, those with a 6-numbered ring bufadienolides. The aglycone portion is essential for pharmacological activity. Its basic structure is a cyclopentanoperhydrophenandrene nucleus in a cis-trans-cis-configuration that makes CG distinct from bile acids, sterol or steroid hormones. Saturation of the lactone ring attenuates activity.

The number of sugar residues linked to the aglycone-portion (1–4) or the hydroxylation of the aglycone markedly influences water and lipid solubility, protein



Cardiac Glycosides. Figure 1 Cardiac glycosides – schematic representation of the Na^+/K^+ -ATPase.

binding, metabolic disposition, duration of action and elimination (Table 1).

Intestinal absorption of digoxin is less complete compared to digitoxin. In order to improve absorption, acetylated- and methylated-digoxin derivatives were developed. Digitoxin is metabolised in hepatic microsomal enzymes and can be cleared independently from renal function. The therapeutic serum level of digoxin is 0.5–2.0 ng/ml and 10–35 ng/ml of digitoxin. Steady state plateau of therapeutic plasma concentrations is reached after 4–5 half-life-times using standard daily doses [5].

Molecular Properties of the Na^+/K^+ -ATPase

The pharmacological receptor of cardiac glycosides is the sarcolemmal Na^+/K^+ -ATPase expressed on most eucaryotic membranes. It was characterised biochemically in 1957 by J. Skou, who was awarded with the Nobel Prize in chemistry in 1997. The sodium

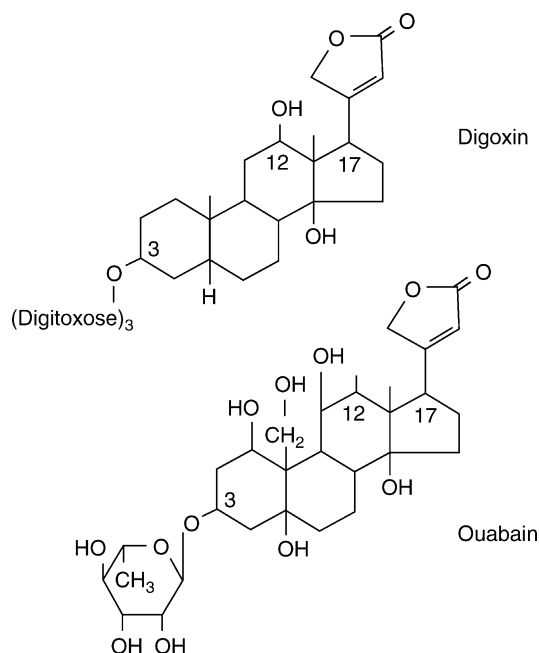
pump belongs to a widely distributed class of P-type ATPases forming a phosphorylated aspartyl residue during the catalytic process. Ions migrate through a narrow “access channel”, and are bound, occluded, and transported by cycling between two different cation-dependent conformations.

Structure and Subunit

The sodium pump is an oligomer composed of two major subunit polypeptides: a catalytic α -unit (MW 110 kD) and a glycosylated β -unit (MW 40–60 kD, depending on degree of glycosylation). The α -unit spans the cell membrane 10-times. Both the NH_2 - and the COOH -termini face the cytoplasm. It is responsible for the catalytic and transport characteristics of the ATPase. The α -unit contains binding sites for cardiac glycosides, ATP, sodium or potassium and the phosphorylation site. The β -unit is a modulator of the transport characteristics and proper membrane insertion of the Na^+/K^+ -ATPase and crosses the membrane once. The COOH -terminus is located at the large ectodomain of the β -subunit. The NH_2 -terminus is exposed to the cytoplasm. A third protein, termed the γ -subunit, can modify the pump activity, yet its physiological significance is unknown.

Isoforms

Na^+/K^+ -ATPase is expressed as several isozymes (4α , 3β subtypes) that are encoded, on different genes. The degree of identity across different species is <92% for $\alpha 1$ and $\alpha 2$ -►isoforms and <96% for the $\alpha 3$ -isoform. Within one species the ►homology of $\alpha 1$ -, $\alpha 2$ - and $\alpha 3$ -isoform is <87%. It is 78% between the $\alpha 4$ - and $\alpha 1$ -isoform. The highest structural variability among the isoenzymes occurs at the NH_2 -terminus, the ouabain-binding site between transmembrane segments 1 and 2, and the region between amino acids 403 and 503. The greatest similarities exist in the cytoplasmic middle region where the ATP binding and phosphorylation sites are located, in the transmembrane hydrophobic regions and in the carboxy-terminal region. Canine vascular smooth muscle utilises alternative RNA processing of the $\alpha 1$ -isoform gene to express a structurally distinct 65 kD isoform, named $\alpha 1\text{-T}$ (truncated) that lacks 40% of the carboxy-terminal sequence, though retains the ATP binding and phosphorylation site. Short variants of the $\alpha 1$ -peptide



Cardiac Glycosides. Figure 2 Cardiac glycosides – molecular structure of digoxin and ouabain. Digoxin contains an additional hydroxy group in position 12 compared to digitoxin.

Cardiac Glycosides. Table 1 Cardiac glycosides

	Ouabain	Digoxin	Digitoxin
Intestinal absorption	3%	60–80%	>98%
Protein binding	3%	25%	90–100%
Renal elimination	>90%	70–80%	60%
Oral daily maintenance dose	–	0.1–0.25 mg	0.07–0.1 mg
Disposition half-time		1.6 days	7–9 days

suggest a cation-selective gate close to the NH₂-terminus. In β -subunits, inhibition of glycosylation by tunicamycin or mutation of the β -subunit renders an active enzyme with catalytically competent sodium pumps and normal affinity for ouabain, yet removal of disulfide bonds abolishes assembly of α/β -subunits. Truncated β -subunits have been described. Different affinities of the isoenzymes for cations and ATP may be essential in adapting cellular Na⁺/K⁺-ATPase activity to specific physiological requirements. Its expression of isoforms is species- and tissue-specific and can be altered during development and by hormones. The $\alpha 1/\beta 1$ -isoenzyme is expressed ubiquitously, whereas the other isoenzymes may play tissue-specific roles. In neuron cells $\alpha 1$ - and $\alpha 2$ -isoforms seem to maintain the basal ionic gradients. The $\alpha 3$ -subunit, because of its low affinity for cations, might serve as a spare pump to help restore the resting membrane potential. The significant role of certain isoforms remains to be established.

The reactivity of the sodium pump isoforms toward ouabain can significantly differ between certain isoenzymes. The $\alpha 1$ -isoform from rat is reported to be 100-fold more resistant to ouabain than $\alpha 2$ - or $\alpha 3$ -isoform. In other species like rabbit, pig, dog or human these differences are not as distinct as in rat, due to a more sensitive $\alpha 1$ -isoform. At least two different types of ouabain binding sites are reported for the Na⁺/K⁺-ATPase [1].

Interactions with the cytoskeleton seem to be responsible for the processing and the targeting of the Na⁺/K⁺-ATPase to the appropriate compartment structures. Protein kinases are considered to play an essential role in modulation of the sodium pump.

Clinical Use

The clinical indications of cardiac glycosides are tachyarrhythmic atrial fibrillation or flutter, as well as

severe heart failure (NYHA III-IV) due to systolic dysfunction. In atrial fibrillation or flutter, the properties of digitalis are the reduction of ventricular heart rate at rest by suppression of the AV-node conduction. By this mechanism digitalis is heart rate limiting, without converting atrial fibrillation into **sinus rhythm**. In heart failure, digitalis leads to a reduction of end diastolic filling pressure and volume, decrease in myocardial wall tension and oxygen consumption, with no evidence of desensitisation or tachyphylaxis. Whereas digitalis enhances vascular tone in healthy subjects by direct action on smooth muscle cells, in heart failure patients baroreflex responsiveness is restored, sympathetic nerve activity is decreased in skeletal muscle and vascular blood flow is increased. Furthermore, cardiac glycosides decrease plasma norepinephrine and renin activity [2].

Clinical Trials

The effect of a digitalis therapy on mortality in patients with chronic heart failure and sinus rhythm receiving diuretics and angiotensin-converting enzyme inhibitors (ACE) has been studied in the DIG (digitalis investigation group) trial. This trial included about 6,800 patients with decreased cardiac ejection fraction (<45%). The follow-up was 37 months. As a result, there was no difference in mortality in both groups, but fewer hospitalisations in the digitalis arm, and a benefit was observed in clinical symptoms in addition to maximum therapeutic regime [3]. Importantly, in a post hoc analysis of the DIG trial a serum digoxin concentration (SDC) of 0.5–0.9 ng/ml was associated with a reduced mortality while there was no effect with SDC ≥ 1 ng/ml [4]. Furthermore, a worsening of heart failure after withdrawal of cardiac glycoside medication in patients with mild to moderate heart failure (NYHA II-III) has been shown in the RADIANCE and PROVE trials.

Cardiac Glycosides. Table 2 Cardiac glycosides

Increased digitalis effects	Mechanism of action	Reduced digitalis effects
Hypocalcemia	Sodium receptor interaction	Hypercalcemia
Hypercalcemia	Sodium receptor interaction	Hypocalcemia
Hypomagnesia	Sodium receptor interaction	Hypermagnesia
Hypothyreosis	Sodium receptor interaction	Hyperthyreosis
Renal failure	Reduced elimination, electrolyte derangements	
Diuretics, laxatives, diarrhoea, emesis	Electrolyte derangements	
	Diminished resorption	Cholestyramine
	Diminished resorption	Neomycin
	Diminished resorption	Sulfasalazine
Erythromycine, tetracycline	Increased resorption	
Quinidine	Diminished renal and nonrenal excretion	
Verapamil, diltiazem, amiodarone	Diminished renal and nonrenal excretion	
	Induction of microsomal enzymes	Phenytoin, rifampin

Interactions and Adverse Effects

Cardiac glycosides have a small ratio of toxic to therapeutic concentration. Possible adverse effects are nausea, vomiting, abdominal pain, diarrhoea, fatigue, headache, drowsiness, colour vision disturbances, sinus bradycardia, premature ventricular complexes, AV-block, bigeminy, atrial tachycardia with AV-Block, ventricular fibrillation. There are several mechanisms relevant for their toxic action (Table 2).

Digoxin is more rapidly eliminated and thereby, possible adverse or toxic effects last for a shorter time.

► Antiarrhythmic Drugs

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Cardiac I_{K1}

Definition

Cardiac I_{K1} is the major K^+ current responsible for stabilizing the resting membrane potential and shaping the late phase of repolarization of the action potential in cardiac myocytes. The name should not be confused with that of an Intermediate conductance calcium-activated K^+ channel, which sometimes is also called I_{K1} .

► Inward Rectifier K^+ Channels

Cariporide

An amiloride analog that inhibits Na^+/H^+ ion exchangers and is under consideration for the prevention of post-ischemic damage.

► Na^+/H^+ Ion Exchangers

Carotinoids

► Retinoids

CART

CART (cocaine- and amphetamine-regulated transcript) is a hypothalamic peptide that inhibits both normal and starvation-induced feeding when injected into cerebral ventricles of rats. CART is co-localized with the anorexigenic peptide α -melanocyte-stimulating hormone in neurons of the arcuate nucleus. Secretion of CART is stimulated by leptin and CART may be an endogenous inhibitor of food intake.

► Psychostimulants

► Orexins

► Appetite Control

Caspases

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Synonyms

Cysteine endopeptidases; Clan CD; Family C14
(► <http://merops.sanger.ac.uk/>)

Definition

Caspases form an evolutionarily conserved family of cysteine endopeptidases. Like the families of clostripains (C11), legumains (C12), gingipains (C25) and separases (C50), the caspase family (C14) belongs to the protease clan CD, which is a small but important group of proteolytic enzymes with a unique α/β fold (► <http://merops.sanger.ac.uk/>). Features common to all members of the caspase family include the catalytic cysteine residue in the active site, and the ability to cleave substrates on the carboxyl side of aspartate residues, hence the name cysteine aspartate specific proteinases or caspases. Many of the caspase-family members are

activated during ►apoptosis, a specialized form of programmed cell death (PCD). In addition, certain caspases also exert non-apoptotic functions in various processes, including cytokine maturation during innate immunity, cell differentiation, proliferation, and NF- κ B activation. Many diseases can be linked to excessive or insufficient caspase activation. Extensive studies of caspases and natural caspase inhibitors in apoptosis and inflammation during pathologies validated the enzymes as attractive drug targets, the inhibition of which could alleviate a variety of human ailments.

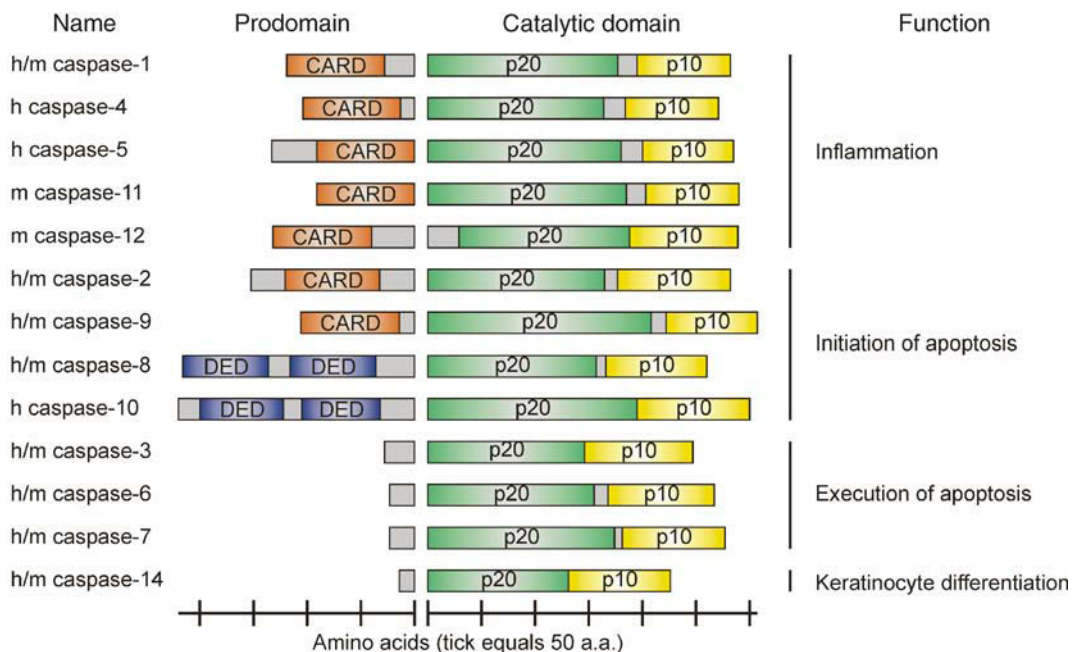
Basic Characteristics

Structure and Activation of Caspases

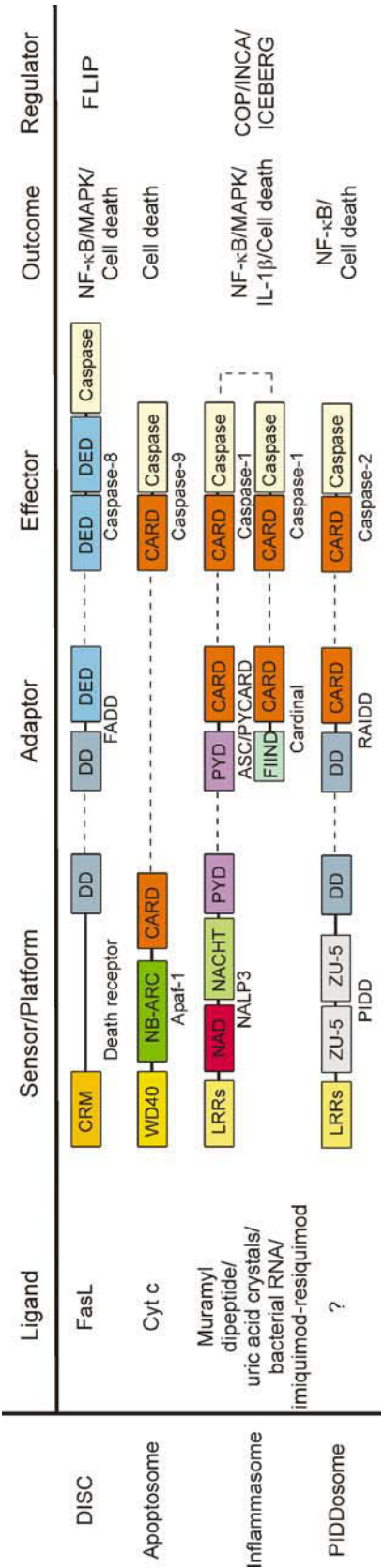
So far ten catalytically active caspases have been reported in mouse (caspase-1, -2, -3, -6, -7, -8, -9, -11, -12, -14) and eleven in human (caspase-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -14) (Fig. 1). Caspases are expressed as inactive proenzymes that contain an amino-terminal prodomain of variable length followed by two domains with conserved sequences: a large subunit (~20 kDa, p20) and a small carboxy-terminal subunit (~10 kDa, p10). Caspases can be divided according to absence (-3, -6, -7, -14) or presence (-1, -2, -8, -9, -10, -11, -12) of an extended prodomain containing protein-protein interaction motifs belonging to the ►death domain (DD) superfamily, in particular the death effector domains (DED) and the caspase activation and recruitment domains (CARD).

The absence or presence of a prodomain determines the fundamental mechanism by which caspases are activated. The proenzyme forms of caspases lacking a prodomain form homodimers that are activated by proteolytic cleavage at aspartate residues in the hinge region between the p20 and p10 subunits. This cleavage causes a conformational change leading to activation of the dimeric protease. The proenzyme forms of the prodomain-containing caspases exist as monomers and are dimerized by homotypic recruitment on specific adaptor proteins in protein complexes; this leads to a conformational change and activation of the procaspases. This results in autoproteolytic cleavage in the hinge regions between p20 and p10 and between the prodomain and p20. Multiprotein complexes that activate caspase-1, -2, -8 and -9 are, respectively, the inflammasomes, the PIDDosome, the death inducing signaling complex (DISC), and the apoptosome (Fig. 2) [1, 2].

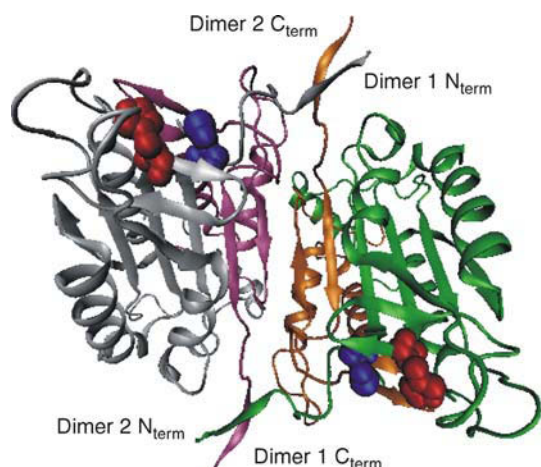
The three dimensional structure of activated dimeric caspases is characterized by the typical caspase fold composed of a central 12-stranded β -sheet plane surrounded by 10 α -helices (Fig. 3). The two dimers align in a head-to-tail configuration, thereby positioning the two active sites at opposite ends of the molecule. The active site spans both the p20 and p10 subunits with His237 and Cys285 (numbering in human procaspase-1), forming a catalytic dyad in the active site of the caspases. Upon substrate binding, the thiolate anion of the catalytic



Caspases. Figure 1 Schematic representation of caspase domain architecture. Illustration of all identified *Homo sapiens* and *Mus musculus* caspases. The prodomain, and the large (p20) and small (p10) subunits are proportionally depicted.



Caspases. Figure 2 Caspase activating complexes. Schematic representation of all described long prodomain caspase activation complexes. Each complex contains essentially three functionally different building blocks: a sensor/platform, an adaptor and an effector in the form of a particular caspase. Some instigating ligands, possible outcomes and regulatory proteins are indicated.



Caspases. Figure 3 Three-dimensional illustration of a caspase. Representation of active caspase-1 adopting the typical caspase fold, consisting of two p20/p10 caspase heteromers folded in a compact cylinder that is dominated by a central six-stranded β -sheet and five α -helices distributed in a three-two configuration on both sides of the plane that is formed by the β -sheet. The subunits of the left heterodimer (dimer 1) are in gray (p20) and purple (p10). The subunits of the right heterodimer (dimer 2) are in green (p20) and orange (p10). The catalytic dyad, His237 (red) and Cys285 (blue), are shown in spacefill.

Cys285 residue participates in a nucleophilic attack on the carbonyl carbon of the scissile amide bond.

Functions of Caspases

Caspases are endopeptidases, meaning that they cleave a substrate very specifically at one or more sites, which results in a loss- or gain-of-function of the substrate. The functionality of the different caspases is thus determined by their substrate spectra (degradome). However, in the case of apoptosis, which is an irreversible cell death process, it is very difficult to distinguish the cleavage events that represent collateral damage and those that contribute to crucial signaling events. From the hundreds of substrates only a few have been shown to contribute to the apoptotic cell death process. However, their proteolytic activity is a central biochemical feature of the apoptotic process during both the initiation and the execution phases. Caspases can be functionally divided into ►**apoptotic initiator caspases** (caspase-2, -8, -9 and -10), which are activated in complexes that sense cellular stress (PIDDosome, DISC, apoptosome), and the ►**apoptotic executioner caspases** (caspase-3, -6, -7), which are activated by the apoptotic initiator caspases and cleave a large number of downstream substrates. Third, the ►**inflammatory caspases** (caspase-1, -4, -5, -11 and -12), with caspase-1 as a prototype, are activated in inflammasome complexes,

and they sense cellular stress, cellular damage and infection. Caspase-1 proteolytically activates inflammatory cytokines, such as pro-IL-1 β and pro-IL-18, and is implicated in their release. In humans, caspase-12 is a catalytically inactive form that is probably involved in dampening the inflammasome-mediated responses by competing for caspase-1 activation. Besides their prominent roles in apoptosis and inflammation, caspases have also been implicated in cell survival, motility, differentiation and proliferation, although it is not clear how these caspases exert their functions without leading to apoptosis. Finally, caspase-14 represents a special case, since it is very specifically expressed in the suprabasal layers of the epidermis and plays a crucial role in water homeostasis of the skin and in protection against UVB radiation.

The different caspases exhibit different substrate specificities for four amino acid stretches ($P_4P_3P_2P_1$) that fit in the substrate-binding cleft of the enzyme. All caspases cleave after an aspartic acid in the P_1 position of the substrate. However, whether a substrate will be recognized and cleaved depends on the accessibility of this cleavage motif on the target protein and on additional interactions between substrate and protease. The use of fluorogenic combinatorial tetrapeptide libraries (acetyl-XXXD-aminomethylcoumarine) led to the identification of the preferential tetrapeptide target sequences. Based on these results, human caspases can be divided into three major groups. Group I caspases (the inflammatory caspases-1, -4 and -5) prefer bulky hydrophobic residues at the P_4 position of the substrate (WEHD). Group II caspases (the downstream apoptotic effector caspases-3 and -7, along with caspase-2) prefer an Asp at the P_4 position (DEXD). Group III caspases (the apoptotic initiator caspases-8, -9, -10 and the apoptotic effector caspase-6) prefer a branched chain aliphatic amino acid residue at the P_4 position (L/VEXD). This specificity formed the basis for the first generation peptide-based synthetic inhibitors of caspases, followed by peptidomimetics (see below).

Regulation of Caspases by Natural Inhibitors

The activation of caspases is tightly controlled by a number of cellular caspase inhibitors [2] [3]. Based on their action mechanisms, natural inhibitors fall in two groups. The first group encompasses some members of the inhibitor of apoptosis protein (IAP) family. Most of the IAP proteins contain BIR motifs and an E3 ligase RING domain. IAPs control caspase expression and activity by a combination of mechanisms involving pseudosubstrate interaction in the catalytic pocket, allosteric inhibition through binding of IAP binding motifs (IBM) on certain caspases, and E3 ligase directed polyubiquitination and degradation of caspases. The most potent and best characterized IAP family member for the inhibition of cell death *in vitro* is XIAP, which mainly targets caspase-3, -7 and -9

with K_i values in the low nanomolar range. Other family members, such as cIAP1, cIAP2 and survivin, cannot directly inhibit caspases in physiological circumstances, because of their high K_i . Caspase inhibitors belonging to the second group prevent caspase activation by competing during the recruitment of inactive procaspases in the caspase activating complexes. Prototypic examples of these caspase-like decoy molecules are the caspase-8 (FLICE) inhibitory proteins (FLIPs) and caspase-1-related CARD-only proteins. Both the short splice variant of cellular FLIP (c-FLIP_s), essentially consisting of a caspase-8-like prodomain, and the long splice variant of cellular FLIP (c-FLIP_L), also containing an inactive caspase-like region, compete with FADD-mediated caspase-8 recruitment and activation in the DISC complex. The three caspase-1-related CARD-only proteins COP, INCA and ICEBERG use a similar mechanism: they compete with ASC/PYCARD-mediated recruitment of procaspase-1 in the inflammasome complex to reduce caspase-1 activation. These CARD-only proteins are the product of recent caspase-1 gene duplications in Hominidae. Remarkably, most of the natural caspase inhibitors were originally identified in viruses, illustrating the importance of these pathways for counteracting pathogens. Caspases are also regulated at the levels of transcription and alternative splicing, by generation of dominant-negative splice-variants that may interfere with heterodimerization of caspases or prevent caspase recruitment in activating complexes.

Involvement of Caspases in Disease

Too strong, too weak, or aberrant activation of one or more caspases is a hallmark of many human ailments. In Table 1 we provide a list of experimental diseases in

which involvement of caspases have been demonstrated using peptide based synthetic caspase substrates, caspase inhibitors or caspase knockout mice.

Drugs

Caspase-1 Inhibitors

Targeting caspases with small-molecule drugs is a very attractive therapeutic strategy for the treatment of several diseases (see overview in Table 2) [2, 3, 4]. Consequently, several pharmaceutical companies invest in the development of specific or pan-caspase inhibitors (PCI). In collaboration, Vertex Pharmaceuticals Inc. and Aventis Pharma AG were the first to enroll a caspase-targeting drug, pralnacasan (VX-740), in clinical trials. Pralnacasan is a caspase-1 specific peptidomimetic pyridazinodiazepine inhibitor (IC₅₀ 1.3 nM) derived from the YVAD tetrapeptide sequence found in a caspase-1 tetrapeptide substrate screening. Pralnacasan acts as a pseudo-substrate that irreversibly binds to the cysteine of the active site. Administered orally, pralnacasan was shown to inhibit caspase-1 *in vivo* by reducing LPS- and carrageenan-induced IL-1 β production in plasma and peritoneal exudates, respectively. Preclinical data obtained in animal models of rheumatoid arthritis (RA) and osteoarthritis (OA), two malignancies displaying elevated IL-1 β and IL-18 levels in cartilage and synovial fluids, provided additional evidence for the therapeutic potential of pralnacasan. Based on these results, clinical trials for pralnacasan were initiated in RA and OA patients. Phase I trials (completed in 1999) proved that the compound was well tolerated and displayed good oral bioavailability. Phase II clinical trials demonstrated that pralnacasan treatment resulted in significant amelioration of RA but not OA. Although promising, clinical trials for RA and

Caspases. Table 1 Diseases associated with inappropriate caspase activation. Contribution of apoptosis (A) and inflammation (I) are indicated

Disease group	Disorder
Excessive caspase activity	
Liver diseases	Alcoholic hepatitis (A), hepatitis B and C (A), non-alcoholic steatohepatitis (A), liver transplantation (A), Wilson's disease (A)
Ischemia-reperfusion damage	Stroke (A,I), cardiac failure (A), transplantation (A)
Neurological diseases	Huntington's disease (A), amyotrophic lateral sclerosis (A,I), Parkinson's disease (A), traumatic brain injury (A,I), glaucoma (A)
Inflammatory and immune diseases	Autoimmune disease (A,I), asthma (A), osteoarthritis (I), rheumatoid arthritis (I), septic shock (A,I), infections (A,I), familial cold auto-inflammatory syndrome (I), Muckle Wells syndrome (I), chronic infantile neurological cutaneous and articular syndrome/neonatal onset multisystemic inflammatory disease (CINCA/NOMID) (I), Crohn's disease (I), gout (I), acute renal failure (A,I)
Decreased caspase activity	
Cancer	Neuroblastoma, leukemia, brain tumors and prostate cancer (A)

Caspases. Table 2 Caspase inhibitors and their clinical status

Compound	Company	Caspase target(s)	Strength of inhibition	Disease target	Clinical status
Pralnacasan (VX-740)	Vertex/Aventis	Caspase-1 (irreversibly)	IC ₅₀ 1.3 nM	Rheumatoid arthritis and osteoarthritis	Phase IIb (suspended)
VX-765	Vertex	Caspase-1 (irreversibly)	IC ₅₀ 0.8 nM	Psoriasis	Phase IIa
IDN-6556	Idun Pharm. (Pfizer)	Caspase-1, -3, -6, -7, -8, -9 (irreversibly)	IC ₅₀ 25 nM	Hepatitis C Liver transplantation	Phase II
M826	Merck-Frosst	Caspase-3 and -7 (reversible)	IC ₅₀ 0.006 μ M (C-3) IC ₅₀ 0.042 μ M (C-7)	Huntington's disease	Pre-clinical
M867	Merck-Frosst	Caspase-3 and -7 (reversible)	K _i 1.4 nM (C-3) K _i 8.9 nM (C-7)	Sepsis	Pre-clinical
Compound 34	Sunesis	Caspase-1	ND	ND	Development

OA were aborted in 2003 due to the development of mild to moderate liver fibrosis in dogs treated for 9 months with high doses of pralnacasan. This was in spite of recent evidence demonstrating the effectiveness of pralnacasan treatment in the acute dextran sulfate sodium (DSS)-induced colitis model, which resembles Crohn's disease (CD) characteristics, and in transient ischemia induced brain damage.

Vertex also put in clinical trial VX-765, another caspase-1-specific, YVAD-derived peptidomimetic that is *in vitro* slightly more potent than pralnacasan (IC₅₀ 0.8 nM). Evaluation of VX-765 in a mouse model of oxazolone-induced dermatitis showed a dose-dependent (10–100 mg/kg) inhibition of ear inflammation. Consequently, VX-765 was enrolled in a 4-week phase IIa safety and pharmacokinetic study for psoriasis. However, Vertex has not communicated any results yet.

Pan Caspase-Inhibitors

Apart from these two Vertex compounds, only one other caspase inhibitor, IDN-6556, has been used in clinical trials. This compound belongs to the class of oxamyl dipeptides and was originally developed by Idun Pharmaceuticals (taken over by Pfizer). It is the only pan-caspase inhibitor that has been evaluated in humans. IDN-6556 displays inhibitory activity against all tested human caspases. It is also an irreversible, caspase-specific inhibitor that does not inhibit other major classes of proteases, or other enzymes or receptors. The therapeutic potential of IDN-6556 was first evaluated in several animal models of liver disease because numerous publications suggested that apoptosis contributes substantially to the development of some hepatic diseases, such as alcoholic hepatitis, hepatitis B and C (HBV, HCV), non-alcoholic steatohepatitis (NASH), and ischemia/reperfusion injury associated with liver transplant. Accordingly, IDN-6556 was tested in a phase I study. The drug was safe and

well-tolerated in healthy adults and patients with mild liver impairment. These patients also displayed statistically significant improvements in alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Analysis of the pharmacokinetic profile demonstrated that IDN-6556 is quickly cleared from the venous circulation after infusion. The effect of IDN-6556 was further explored in a phase II study involving 80 patients with chronic HCV and 25 patients with other liver diseases, including NASH and HBV. IDN-6556 significantly reduced ALT and AST levels in HCV patients. In addition, IDN-6556 was also successfully tested in patients experiencing liver transplantation or suffering ischemia-reperfusion injury after lung transplantation. In conclusion, broad-spectrum caspase inhibitors, such as IDN-6556, are promising drugs for the treatment of several disorders.

Pre-Clinical Caspase Inhibitors

Aside from the three above-mentioned compounds that have entered clinical trials, many other compounds are being evaluated in different animal models. Two of the more promising inhibitors, developed by Merck-Frosst, are M826 and M867. These pyrazinone monoamides selectively and reversibly inhibit effector caspases-3 and -7. Both M826 and M867 demonstrated excellent anti-apoptotic activities *in vitro* as well as in several *in vivo* models. For example, M826 displayed a neuronal protective effect in malonate induced ischemic injury in rat a model for Huntington's disease. M867, when given as a continuous infusion, dose-dependently inhibited caspase-3 activity in a rat model of sepsis induced by cecal ligation and perforation. These findings demonstrated for the first time the potential therapeutic benefit of using a selective and reversible caspase-3 and -7 inhibitor to treat acute injuries. Recently, researchers at Sunesis described a different approach, namely targeting a cysteine in the

caspase's allosteric site with a drug that prevents the caspase from adopting the active conformation. In this way they identified their "compound 34," which forms a thiol linkage in the dimeric interface of caspase-1. Sunesis has not provided any information on *in vivo* results so far.

Inhibitors of Natural Caspase Inhibitors as Anti-Cancer Drugs

Chemotherapeutic drugs and radiotherapy kill cancer cells largely by inducing apoptosis [5]. However, most human cancers show considerable resistance to apoptosis. Elevated levels of IAPs (see above), which inhibit caspases, are found in many human cancers and have been associated with poor prognosis. Targeting IAPs presents a promising novel approach to potentiate the effect of chemotherapy and radiotherapy on cancer cells. Recent findings indicate that inhibition of the caspase inhibitor XIAP may prove to be a successful strategy to overcome apoptosis resistance of human cancers. The mitochondrial proteins Smac/DIABLO and Omi/HtrA2 are released from the intermembrane space during apoptosis and contain an amino-terminal IAP binding motif (IBM) that can inhibit the interaction between XIAP and caspase, thereby relieving the inhibitory activity. Peptide inhibitors based on the IBM motif were developed. These inhibitors, when used in combination with chemotherapeutics, γ -irradiation or TRAIL, sensitize several human cancer cell lines and neuroblastoma xenografts for apoptosis. Based on the NMR structure of the IBM motif complexed with XIAP, a novel series of XIAP peptidomimetic antagonists were developed by Idun Pharmaceuticals (now Pfizer Inc). These XIAP small-molecule antagonists "XAntag" were tested both *in vitro* and *in vivo* against a panel of human pancreatic cancer cell lines and showed significant anti-cancer activity, especially in combination therapy with TRAIL or γ -radiation. In conclusion, targeting the caspase inhibitor XIAP by small molecules is a promising new approach to potentiate the efficacy of several anti-cancer therapies.

List of Abbreviations

ALT, alanine aminotransferase
 ASC, apoptosis-associated speck-like
 protei containing a CARD
 AST, aspartate aminotransferase
 CARD, caspase activation
 and recruitment domains
 CD, Crohn's disease
 COP, CARD-only protein
 DD, death domain
 DED, death effector domains
 DIABLO, direct IAP-binding protein with
 low pI

DISC, death inducing signaling complex
 DSS, dextran sulfate sodium
 FLIP, caspase-8 (FLICE) inhibitory
 protein
 HBV, hepatitis B
 HCV, hepatitis C
 IAP, inhibitor of apoptosis protein
 IBM, IAP binding motifs
 INCA, inhibitory CARD
 NASH, non-alcoholic steatohepatitis
 PCD, programmed cell death
 PCI, pan-caspase inhibitor
 OA, osteoarthritis
 RA, rheumatoid arthritis
 Smac, second mitochondria-derived
 activator of caspases
 TRAIL, tumor necrosis factor-related
 apoptosis-inducing ligand.

► Tumor Necrosis Factor (TNF)

► Apoptosis

► Neurodegeneration

► Non-viral Peptidases

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Cataplexy

An episodic sudden loss of postural muscle tone and function, ranging from slight weakness to complete body collapse, due to imbalanced descending monoaminergic and cholinergic modulation of motoneurons. In human ►[narcolepsy](#) it is, typically triggered by emotionally arousing, appetitive or pleasurable stimuli, such as joy and laughter, or food presentation in animals.

► Orexins

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited heart disorder induced by an intense adrenergic activity (physical or emotional stress), often resulting in syncope or sudden death with no structural abnormalities. The ECGs of patients with CPVT show typical pattern of bidirectional ventricular tachycardia during exertion but are unremarkable at rest. Two genes are currently associated with CPVT: cardiac type ryanodine receptor (RyR2) and calsequestrin (CASQ2).

► Ryanodine Receptor

Catecholamines

Catecholamines are biogenic amines with a catechol (*o*-dihydroxy-benzol) structure. They are synthesized in nerve endings from tyrosine and include dopamine, noradrenaline (norepinephrine) and adrenaline (epinephrine).

- β -Adrenergic System
- α -Adrenergic System
- Catechol-O-Methyltransferase and its Inhibitors

Catechol-O-Methyltransferase

PEKKA T. MÄNNISTÖ

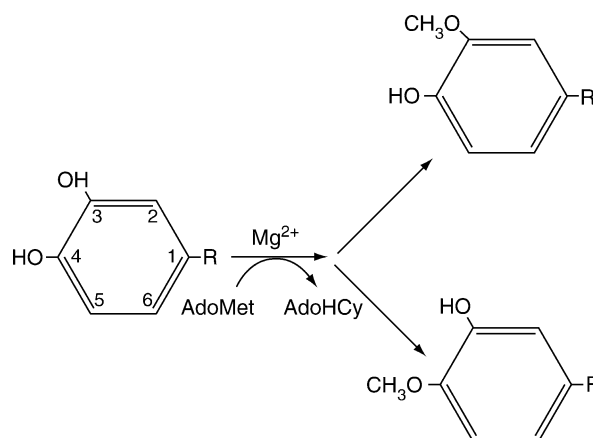
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Synonyms

COMTase; COMT; EC 2.1.1.6

Definition

Catechol *O*-methyltransferase (COMT) is a widespread enzyme that catalyzes the transfer of the methyl group of ► [S-adenosyl-l-methionine](#) (AdoMet) to one of the phenolic group of the catechol substrate (Fig. 1). High COMT activity is found in the liver, kidney and gut wall

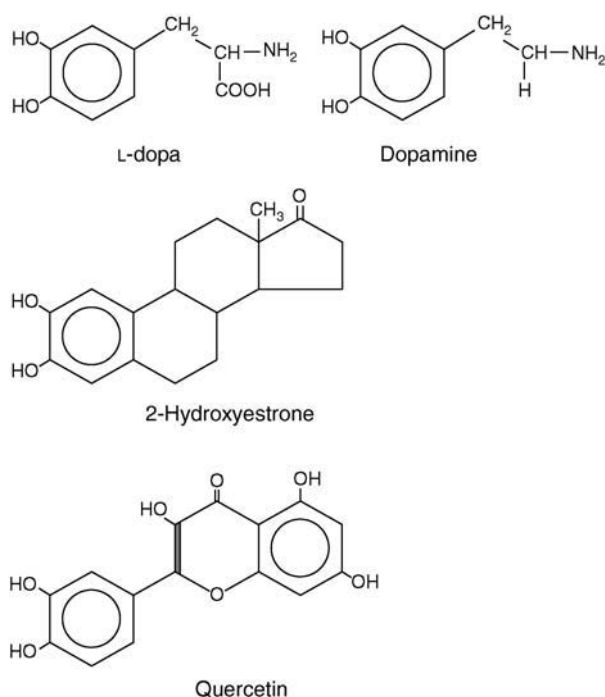


Catechol-O-Methyltransferase. Figure 1 The basic function of COMT. Enzymatic *O*-methylation of the catechol substrate to 3-methoxy (major route) or 4-methoxy (minor route) products in the presence of Mg²⁺ and S-adenosyl-methionine (AdoMet).

[1,2]. A single *COMT* gene codes for two separate enzymes, soluble (S-COMT) and membrane bound (MB-COMT) forms. S-COMT contains 221 amino acids. MB-COMT has an additional amino-terminal extension of 43 (rat) or 50 (man) amino acids. The hydrophobic 17 and 24 amino acid residues in rat and man, respectively, form an α -helical transmembrane domain that serves as a membrane anchor; otherwise the two proteins are similar. MB-COMT is associated with intracellular membranes, not with cell membranes. Also S-COMT is strictly intracellular locating either in cytoplasm or nucleus. Synthesis of recombinant S-COMT in *Escherichia coli* and MB-COMT in insect cells, using baculovirus vectors, has helped to clarify the biochemistry, physiology and pharmacology of COMT [3]. The active site of COMT consists of the AdoMet-binding domain and the catalytic site. S-COMT is abundant in peripheral tissues while MB-COMT prevails in the brain. The catalytic site is formed by a few amino acids that are important for the binding of the substrate, water and Mg²⁺, and for the catalysis of *O*-methylation. The Mg²⁺, which is bound to COMT only after AdoMet binding, improves the ionization of the hydroxyl groups. The lysine residue (Lys144), which accepts the proton of one of the hydroxyls, acts as a general catalytic base in the nucleophilic methyl transfer reaction. Mice lacking totally COMT (knock-out mice) or only S-COMT have been developed. These animals behave and breed quite normally suggesting that COMT is not a vital enzyme [3].

Mechanism of Action

COMT *O*-methylates ► [catecholamines](#) and other compounds having a catechol structure including catecholestrogens (Fig. 2). The two isoforms of



Catechol-O-Methyltransferase. Figure 2 Some substrates of COMT.

COMT may have distinct roles: MB-COMT, a high-affinity isoform of COMT, is supposed to be partially responsible for the termination of dopaminergic and noradrenergic synaptic neurotransmission. S-COMT, on the other hand, is a high capacity enzyme isoform being mainly responsible for the elimination of biologically active or toxic, particularly exogenous, catechols and some hydroxylated metabolites. During the first trimester of pregnancy, COMT present in the placenta protects the developing embryo from hydroxylated compounds. COMT also acts as an enzymatic detoxicating barrier between the blood and other tissues, shielding against the detrimental effects of hydroxylated xenobiotics. COMT may serve some unique or indirect functions in the kidney and intestine tract by modulating the dopaminergic tone. The same may be true in the brain: COMT activity may regulate the amounts of active dopamine particularly in frontal cortical areas of the brain and therefore be associated with mood and other mental processes. COMT has a genetic one-nucleotide polymorphism causing valine amino acid to be changed to methionine leading to thermolability even at +37°C and a decrease of COMT activity to one third to one fourth. Low COMT activity is connected to improved cognitive functions, probably due to increased dopaminergic activity. However, from population studies there is some evidence that certain psychiatric illnesses (e.g. schizophrenia, rapidly fluctuating depression) and even breast cancer in

postmenopausal women may be slightly increased in people having low COMT activity [3].

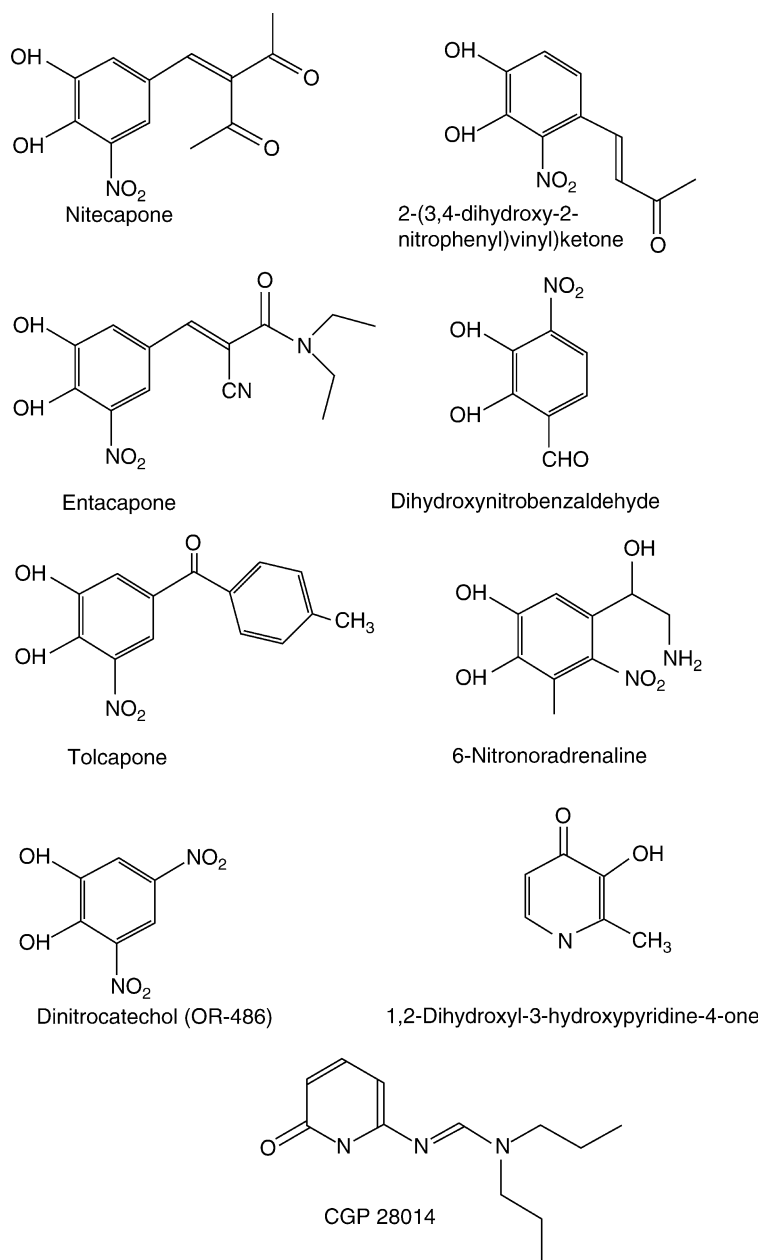
Early COMT inhibitors, like gallates, tropolone and U-0521 (3',4'-dihydroxy-2-methyl-propiofenone) have IC_{50} and K_i values in the micromolar range or higher but may still be practical in vitro tools. However, owing to unfavourable pharmacokinetics and toxicity their clinical use is not possible [1].

Second generation COMT inhibitors were developed by three laboratories in the late 1980s. Apart from CGP 28014, nitrocatechol is the key structure of the majority of these molecules (Fig. 3). The current COMT inhibitors can be classified as follows: (i) mainly peripherally acting nitrocatechol-type compounds (entacapone, nitecapone, BIA 3-202), (ii) broad-spectrum nitrocatechols having activity both in peripheral tissues and the brain (tolcapone, Ro 41-0960, dinitrocatechol, vinylphenylketone), and (iii) atypical compounds, pyridine derivatives (CGP 28014, 3-hydroxy-4-pyridone and its derivatives), some of which are not COMT inhibitors in vitro but inhibit catechol *O*-methylation by some other mechanism. The common features of the most new compounds are excellent potency, low toxicity and activity through oral administration. Their biochemical properties have been fairly well characterized. Most of these compounds have an excellent selectivity in that they do not affect any other enzymes studied [2, 3].

Tolcapone and Ro 41-0960 are longer acting than entacapone and nitecapone in in vivo studies in rats. All types of COMT inhibitors prolong the L-dopa-induced turning behaviour of rats having unilateral nigral lesions (Fig. 4). This has generally been used as a reliable rat model of Parkinson's disease. It is noteworthy that the peripherally acting compound entacapone is practically as effective as the broad-spectrum compound tolcapone. This suggests that the majority of the beneficial action is peripheral in origin, evidently through enhanced bioavailability of L-dopa. Statistics: * $P < 0.05$ and ** $P < 0.01$ versus corresponding controls (data from [4]).

Clinical Use (Including Side Effects)

The main clinical use of COMT inhibitors is as adjunct (or additional adjunct) in the therapy of Parkinson's disease. The standard therapy of Parkinson's disease is oral L-dopa (as a drug levodopa) given with a dopa decarboxylase (DDC) inhibitor (e.g. carbidopa and benserazide), which does not reach the brain. When the peripheral DDC is inhibited, the concentration of 3-*O*-methyl-dopa (3-OMD), a product of COMT, in plasma is many times that of L-dopa. Since the half-life of 3-OMD is about 15 h, compared to about 1 h for L-dopa, the concentration of 3-OMD remains particularly high during chronic therapy, especially if new slow release L-dopa preparations are used. A triple therapy (L-dopa plus DDC inhibitor plus COMT-inhibitor) will

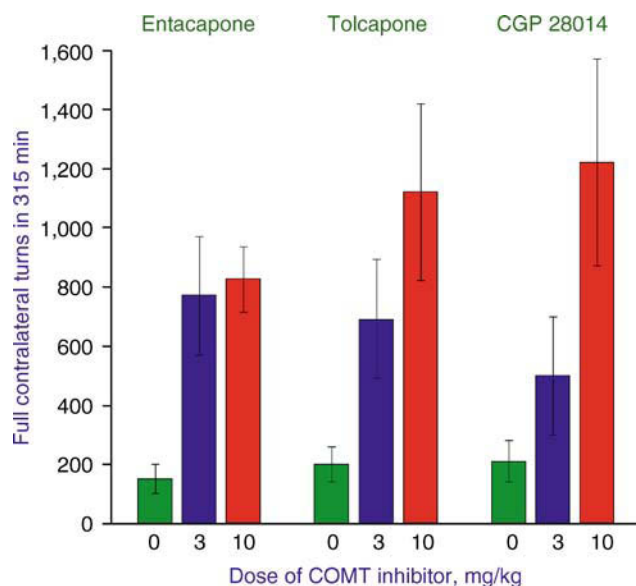


Catechol-O-Methyltransferase. Figure 3 Chemical structures of some inhibitors of catechol O-methylation.

evidently substitute the present double therapy in the coming years. A fixed combination preparation containing all three active drugs is indeed getting very popular worldwide.

COMT inhibitors rescue ►**L-DOPA** and improve the brain entry of L-dopa by decreasing 3-OMD formation in peripheral tissues. The dose of L-dopa could be decreased, compared with the present combination therapy. Dose interval of L-dopa could also be prolonged. Further, COMT inhibitors should decrease fluctuations of dopamine formation in the brain.

Clinical studies, available only for entacapone and tolcapone, support preclinical findings. A dose-dependent (100–800 mg) inhibition of the COMT activity of the erythrocytes can be seen after nitrocatechols. However, effective and sufficient dose levels of both entacapone and tolcapone, given concomitantly with L-dopa and DDC inhibitors to patients with Parkinson's disease, appear to be 100–200 mg. However, the treatment strategies of entacapone and tolcapone differ: entacapone is a short-acting compound that is given with each dose of L-dopa, and COMT activity may even



Catechol-O-Methyltransferase. Figure 4 Rat model of Parkinson's disease. Comparison of entacapone, tolcapone and CGP 28014 in the rat turning model of Parkinson's disease [4].

recover between the doses. Tolcapone, as a longer-acting compound, is given three times a day, and the aim is to keep COMT inhibited most of the time [2, 3].

Since several ►**adrenergic drugs**, having a catechol structure, are also COMT substrates, it is possible to prolong or even potentiate in some cases their actions by COMT inhibitors. Such drugs include bronchodilating compounds (adrenaline, isoprenaline, rimiterol), dopamine agonists (dobutamide, fenoldopam, apomorphine) and antihypertensive drugs (α -methyl-dopa). It is possible to potentiate interactions with endogenous catecholamines during stress and exercise and adverse drug interactions with, e.g. exogenous ►**noradrenaline** and the drugs mentioned above. Fortunately, interaction studies in animals and man have not been able to substantiate this threat. Evidently, the capacity of S-COMT in the peripheral tissues is so high that only a minor general COMT inhibition can ever been achieved. Oestrogens are easily hydroxylated to catecholestrogens, which serve as COMT substrates. The consequence of preventing the major metabolic pathway of catecholestrogens by COMT inhibitors requires further studies; it is possible that quinone-forming pathways are activated [2,3].

In patients having Parkinson's disease, both entacapone and tolcapone potentiate the therapeutic effect of L-dopa and prolong the daily ON time by 1–2 h. In the clinic, COMT inhibitors have been well tolerated, and the number of premature terminations has been low. In general, the incidence of adverse events has been higher in tolcapone-treated patients than in entacapone-treated patients. The main events have comprised of dopaminergic and gastrointestinal problems [2, 3].

Dopaminergic overactivity causes an initial worsening of levodopa-induced ►**dyskinesia**, nausea, vomiting, orthostatic hypotension, sleep disorders and hallucinations. Tolcapone has been associated with diarrhoea in about 16–18% of cases and entacapone in less than 10% of cases. Diarrhoea has led to discontinuation in 5–6% of patients on tolcapone and in 2.5% of those on entacapone. Urine discolouration to dark yellow or orange is related to the colour of COMT inhibitors and their metabolites. Elevated liver transaminase levels are reported in 1–3% of patients treated with tolcapone but very rarely, if at all, in patients treated with entacapone. Three cases of acute, fatal fulminant ►**hepatitis** have been described in association of tolcapone where more than 100,000 patients have been treated. In addition, a few potentially fatal neurological adverse reactions, including ►**neuroleptic-like malignant syndrome**, have been described. Because of these serious adverse drug reactions, tolcapone marketing was temporarily suspended in Europe and Canada in 1999. Now tolcapone is again available in most markets but certain precautions and a regular follow-up of liver function need to be obeyed. So far, no restrictions of the use of entacapone have been proposed [2, 3].

Use of COMT Inhibitors in Positron Emission Tomography

An additional benefit of COMT inhibitors can be found in positron emission tomography (PET) studies. In PET, using 6-[18 F]-fluoro-L-dopa (6-FD) to visualize the brain dopamine metabolism, the peripheral formation of 3-O-methyl-6-[18 F]-fluoro-L-dopa (3-OMFD) by COMT is harmful. 3-OMFD contaminates the brain radioactivity analysed since it is easily transported like 3-OMD to the

brain. COMT inhibition would reduce the formation of 3-OMFD and improve PET analysis. Since 3-OMFD formation continues in the brain, central COMT inhibition may be assumed to offer a further advantage [5].

- ▶ α -Adrenergic System
- ▶ β -Adrenergic System
- ▶ Synaptic Transmission
- ▶ Anti-Parkinson Drugs

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Cathepsins

Cathepsins are intracellular proteinases that reside within lysosomes or specific intracellular granules. Cathepsins are used to degrade proteins or peptides that are internalised from the extracellular space. Some cathepsins such as cathepsin-G or cathepsin-K may be released from the cell to degrade specific extracellular matrix proteins. All cathepsins except cathepsin-G (serine) and cathepsin-D (aspartyl) are cysteine proteinases.

- ▶ Non-viral Peptidases

Cationic Proteins

Basic (pH) proteins directed against pathogens. Examples are the major basic protein from mast cells, the eosinophilic cationic proteins from eosinophils, and ▶ **defensins** from epithelial cells and neutrophilic granulocytes.

- ▶ Bronchial Asthma

Causalgia

Causalgia is burning pain evoked by the activation of sympathetic efferent fibres. The likely mechanism underlying this syndrome involves ectopic expression of α -adrenoceptors on nociceptive afferents following peripheral injury or disease.

- ▶ Pain and Nociception

Caveolae

Caveolae are invaginations of the plasma membrane. They contain the protein caveolin and are rich in certain phospholipids. Similar to coated pits, they bud off internally forming endocytic vesicles. Caveolae play an important role in the internalization of certain cell surface receptors.

- ▶ Lipid Modifications

CCK

Cholecystokinin.

- ▶ Appetite Control

CD26

CD26 is a synonym for dipeptidylpeptidase IV.

- ▶ Non-viral Peptidases
- ▶ Neuropeptide Y

CDK

Cyclin-dependent kinase.

- ▶ Cell Cycle Control

CDR

Complementarity determining regions (CDR's): Loop regions within antibodies and the T cell receptor that are highly variable in composition and structure. The CDR's contribute largely to the specificity of antigen recognition. In antibodies/T cell receptors the heavy/beta or light chain/alpha variable domains each contain three CDR's.

► T Cell Receptors

β-cell

► Pancreatic β-cell

Cell Adhesion Molecules

Integrins, selectins, cadherins, claudins and other cell adhesion molecules are involved in the interaction of cells with other cells or with extracellular matrix components. Some of them also serve as “receptors” by inducing outside-in or additional inside-out signaling.

- Transmembrane Signaling
- Anti-integrins, Therapeutics and Diagnostic Implications

Cell-cycle Arrest

Cell-cycle arrest is the status of a cell population in which progression through the cell division cycle has been halted as a result of checkpoint activation.

► Cell Cycle Control

Cell Cycle Checkpoints

Mechanisms controlling the cell cycle transitions. Cell cycle checkpoints are signal transduction pathways comprising a sensor detecting cellular damage or improper cell cycle transition, a signal transduction cascade for amplifying the signal generated by the sensor, and a target which initiates gene transcription leading to cell cycle arrest, initiation of repair programs, or apoptosis. Alternatively, the target can induce cell cycle arrest directly. One distinguishes DNA replication, DNA damage, and spindle assembly checkpoints.

► Cell Cycle Control

Cell Cycle Control

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Synonyms

Cell division cycle; Cell proliferation; Cell multiplication

Definition

The eukaryotic somatic cell cycle is defined by a sequential order of tasks a dividing cell has to complete: it must replicate its DNA, segregate its chromosomes, grow, and divide. The cell cycle can be divided into four discrete phases. ► **DNA replication** is restricted to S phase (DNA synthesis phase), which is preceded by a gap phase called G1 and followed by a gap phase called G2. During mitosis (M phase) the sister chromatids are segregated into two new daughter nuclei and mitosis is completed by the division of the cytoplasm termed ► **cytokinesis** (Fig. 1).

Basic Mechanisms

Upon mitogenic stimuli, cells can enter the cell cycle, which lasts in the case of a typical dividing human somatic cell for about 24 h. During a single cell cycle, a cell accomplishes four tasks: growth, DNA replication, *chromosome segregation*, and cell division. How a cell regulates the cell cycle and how tumor cells can override these regulatory mechanisms, leading to uncontrolled proliferation, is still insufficiently understood and the subject of intense research. However, it has become clear in recent years that the basic mechanisms of cell cycle

control are remarkably conserved throughout evolution and involve the following mechanisms:

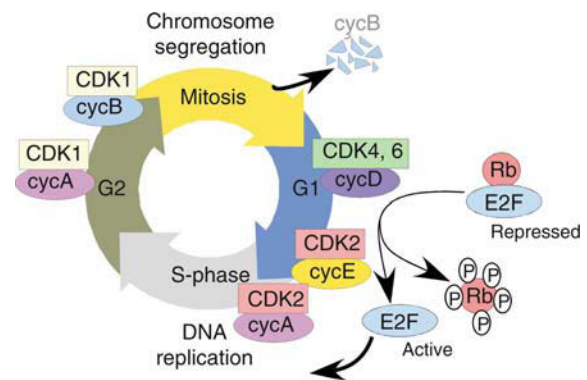
1. reversible protein phosphorylation
2. regulated ►ubiquitin-dependent protein degradation
3. transcriptional control mechanisms
4. regulation by CDK inhibitors
5. ►cell cycle checkpoint mechanisms

Cell Cycle Regulation by Protein Phosphorylation

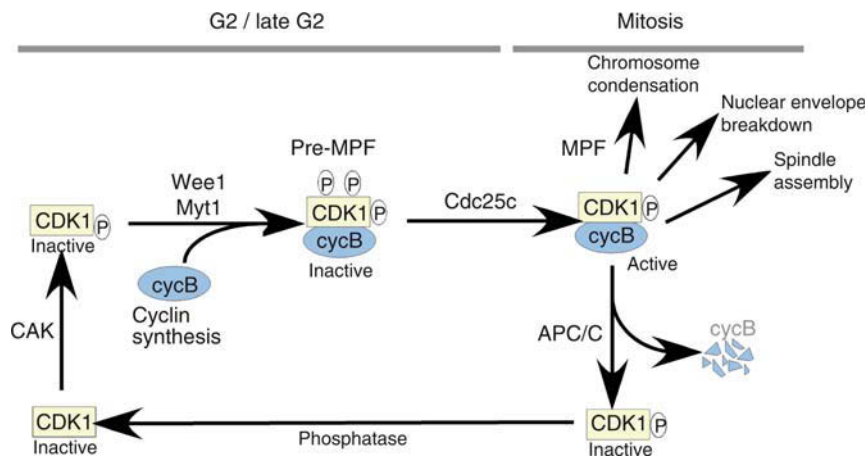
The discovery of a dominant activity designated as the “mitosis (or maturation) promoting factor” (MPF), which drives interphase cells into mitosis led to the idea that certain oscillating activities are responsible for the initiation of mitosis and for driving the different phases of the cell cycle. Biochemical purification of MPF from sea urchin eggs, frog eggs, and mammalian cells as well as a large number of genetic experiments in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* using various “cell division cycle (cdc) mutants” revealed that MPF represents a heterodimeric protein kinase consisting of a so-called cyclin dependent kinase (CDK1, cdc2, or p34cdc2) and its regulatory subunit now known as cyclin B. The principle of a heterodimeric kinase consisting of a CDK and a cyclin subunit was subsequently found for multiple CDKs. Different CDKs including CDK1, 2, 4, and 6 and different cyclins including cyclin A, B, E, and D act as key players for the regulation of different cell cycle transitions (Fig. 1).

CDK1–cyclin B kinase represents the key trigger for entry into mitosis. In late G2, cyclin B binds to CDK1 leading to a preactivated kinase complex. After binding of cyclin B to CDK1, this complex is kept inactive by phosphorylation of the CDK1 protein. First, an activating phosphorylation at threonine-161 is carried

out by a CDK activating kinase (CAK) followed by two inhibitory phosphorylations at residues threonine-14 and tyrosine-15 (in mammals) by the kinases Myt1 and Wee1, respectively. This inactive CDK1–cyclin B complex is called the preMPF and can be activated just prior to mitosis by removing both inhibitory phosphorylation sites by the action of the dual specificity phosphatase Cdc25C (Fig. 2). The inactivating Kinases Myt1 and Wee1 as well as the activating phosphatase Cdc25C are themselves part of a complex regulatory network involving other upstream kinases and phosphatases. Importantly, they are also critical targets for cell cycle checkpoint control mechanisms (see below).



Cell Cycle Control. Figure 1 Cell cycle regulation by Cyclin dependent kinases (CDKs). Different cyclins bound to different CDKs promote the transition from one cell cycle phase into another. CDK-dependent phosphorylation of Rb is required to release active E2F transcription factors, which promotes entry into S phase.



Cell Cycle Control. Figure 2 Regulation of CDK1 activity at the G2/M transition. The pre-MPF complex consisting of CDK1 and cyclin B is activated by Cdc25C-mediated dephosphorylation at the G2/M border. Active CDK1 is required for entry into mitosis and progression through mitosis. CDK1 is inactivated at the end of mitosis by ubiquitin-dependent protein degradation of cyclin B.

The activated CDK1–cyclin B kinase finally phosphorylates a large number of proteins most of which are not well characterized, leading to chromosome condensation, nuclear envelope breakdown, spindle assembly, and ►[chromosome segregation](#).

Cell Cycle Regulation by Ubiquitin-Mediated Protein Degradation

Cyclins are positive regulatory subunits of CDKs. They represent a family of oscillating proteins that are able to bind to and activate specific CDKs in a cell cycle dependent manner. Each cyclin has certain specificities to activate particular CDKs. Cyclins exhibit cell cycle dependent protein levels, which are the result of transcriptional regulation, stability of their mRNAs, and – maybe most importantly – their protein stability. The cyclin B gene is transcriptionally downregulated during G1 phase and becomes activated during S and G2. Cyclin B protein accumulates continuously throughout S and G2 phase, binds and activates CDK1 in late G2 and during the early stages of mitosis. At the metaphase to anaphase transition cyclin B levels drop dramatically, leading to an irreversible inactivation of CDK1, which is required for exit from mitosis (Fig. 2). The decrease in cyclin B levels after the metaphase to anaphase transition is due to regulated ubiquitin-mediated protein degradation (►[ubiquitin/proteasome](#)) by the 26S proteasome. The ubiquitination of cyclin B is a prerequisite for its proteasome-dependent degradation and requires three enzymatic activities including a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3). UbcH5 and UbcH10 are likely the ubiquitin-conjugating enzymes involved in cyclin B ubiquitination while the relevant E3 ligase activity is part of a 1.5 MDa complex known as the *anaphase-promoting complex* or *cyclosome* (APC/C) [1]. During mitosis, the APC/C is specifically activated by coactivator proteins of the Cdc20/Cdh1 family, which also contribute to the recognition of substrate proteins containing a specific sequence motif termed the destruction box (D-box). In addition to mitotic cyclins, the APC/C is involved in the destabilization of various other mitotic target proteins including securin whose degradation at the metaphase to anaphase transition is required for sister chromatid separation. Thus, the APC/C controls the progression and the exit from mitosis. In addition, APC/C-dependent degradation of cyclins during the G1 phase is required to prevent a premature entry into S phase. Moreover, the APC/C controls the degradation of regulators of S phase to regulate replication origin assembly and the APC/C is active in nondividing and differentiated cells suggesting that it has functions beyond the cell cycle [1].

In addition to protein proteolysis during mitosis, ubiquitin-mediated protein degradation (►[ubiquitin/proteasome](#)) is also required at the G1 to S transition

and during S phase. An ubiquitin ligase complex termed SCF (*Skp1-Cdc53-F-box protein*), which is distinct but related to the APC/C functions to ubiquitinate several substrate proteins including the CDK inhibitor p27Kip1, cyclin E, and the transcription factor E2F-1. SCF mediated ubiquitination is essential for proper G1 to S transition.

Together, it is now well recognized that ubiquitin-mediated protein degradation is an important regulatory mechanism throughout the cell cycle for an irreversible inactivation of various cell cycle regulators, including cyclins, mitotic regulators, CDK inhibitors, and transcription factors.

G1/S Transition and Transcriptional Control

The commitment of cells to enter S phase occurs at the so-called restriction point during late G1 phase. Mitogenic growth factors are required for cells to enter the cell cycle. However, beyond the restriction point, mitogenic growth factors are no longer required to complete the cell cycle. This transition is, at least in part, driven by G1 cyclins activating CDK2, 4, and 6. When cells enter the cell cycle from quiescence (G0), cyclin D (different types: D1, D2, D3), cyclin E, and cyclin A are synthesized sequentially. Cyclins A and E preferentially activate CDK2 whereas D type cyclins activate either CDK4 or CDK6 (Fig. 1). The regulatory principles described for CDK1–cyclin B at G2/M apply similarly to the cyclin–CDK complexes functioning at the G1/S transition. For example, activation of CDK2–cyclin A/E and CDK4/6–cyclin D complexes require an activating and two inhibitory phosphorylations, which are removed at or near G1/S phase by the Cdc25A, a phosphatase related to Cdc25C.

When cells enter the cell cycle, cyclin D dependent CDK4 and CDK6 activity is first detected in mid-G1 phase and increases when cells approach the G1/S transition. The most recognized function of cyclin D containing CDKs is phosphorylation of the retinoblastoma protein (Rb). Rb functions as a transcriptional repressor of the E2F family of DNA-binding transcription factors (E2F). E2F binding sites are found in promoters of many genes that are important for ►[DNA replication](#) and therefore for initiation of S phase (e.g., *DHFR*, *DNA polymerase α* , *thymidine kinase*, *thymidylate synthase*, *cyclin E*, and others). In early to mid-G1 phase, Rb is hypophosphorylated and bound to E2F repressing its transcriptional activity, thereby inhibiting entry into S phase [2]. When G1 phase progresses, cyclin D dependent CDK activity increases, followed by an increase in cyclin E and cyclin A dependent kinase activity, leading to hyperphosphorylation of Rb. The phosphorylated form of Rb can no longer bind to E2F and its transcriptional activity is restored. This leads to the activation of E2F target genes allowing initiation of S phase (Fig. 1) [2].

During S phase, the genome has to be replicated completely and only once per cell cycle to maintain genomic integrity. ►**Replication** is initiated from multiple origins on the different chromosomes at specific DNA sequences, the autonomously replicating sequences (ARS). According to a current model, a prereplication complex is formed during G1 phase. This complex includes the loading Cdc6, Orc, and Mcm proteins onto the chromatin. G1-Chromatin, “licensed” for replication, is then guided to S phase by phosphorylation of CDKs active at the G1/S transition. Upon entry into S phase, the prereplication complex is then rearranged, accompanied by the recruitment of chain elongation factors, and an establishment of replication forks. DNA replication induces the dissociation of MCM proteins from chromatin and CDK2 activity during S phase prevents its reloading onto chromatin, thereby restricting the initiation of replication to only once per cell cycle.

G1 Control by CDK Inhibitors

CDK activities can be regulated by CDK inhibitors (CKIs). CKIs are small proteins which can be subdivided into two distinct groups: the INK4 proteins (*inhibitors of CDK4*) including p16INK4a, p15INK4b, p18INK4c, and p19INK4d, named by their ability to bind and to inactivate the catalytic subunit of CDK4 and CKD6 [3]. This group is opposed by the Kip/Cip family of CDK inhibitors including p27Kip1, p21Cip1, and p57Kip2, which display a broader range of substrate specificity. The signals that induce synthesis of the INK4 proteins remain unclear. However, p15INK4b is induced by TGF- β signaling where it plays an essential role in mediating the TGF- β triggered G1 arrest. p16INK4a accumulates during ►**senescence**, thereby contributing to the G1 block. p18INK4c and p19INK4d might have roles during terminal differentiation [3].

Kip/Cip proteins are induced upon stress signals (e.g., after genotoxic damage) and function as biological brakes of the cell cycle. The best example investigated is the transcriptional activation of the *p21Cip1* gene by p53 (see below). Accumulated p21Cip1 binds directly to CDK2–cyclin A/E and inhibits the kinase activity. Thus, p21Cip1 prevents entry into S phase in response to cellular damage [3].

CKIs are important tumor suppressor genes. Disruption of *p16INK4a* is a common event in human cancer and low levels of p27Kip1 caused by enhanced protein degradation appear to be associated with tumorigenesis and poor prognosis. This is also underlined by studies in *p27Kip1* heterozygous mice, which show a haploinsufficiency for tumor suppression.

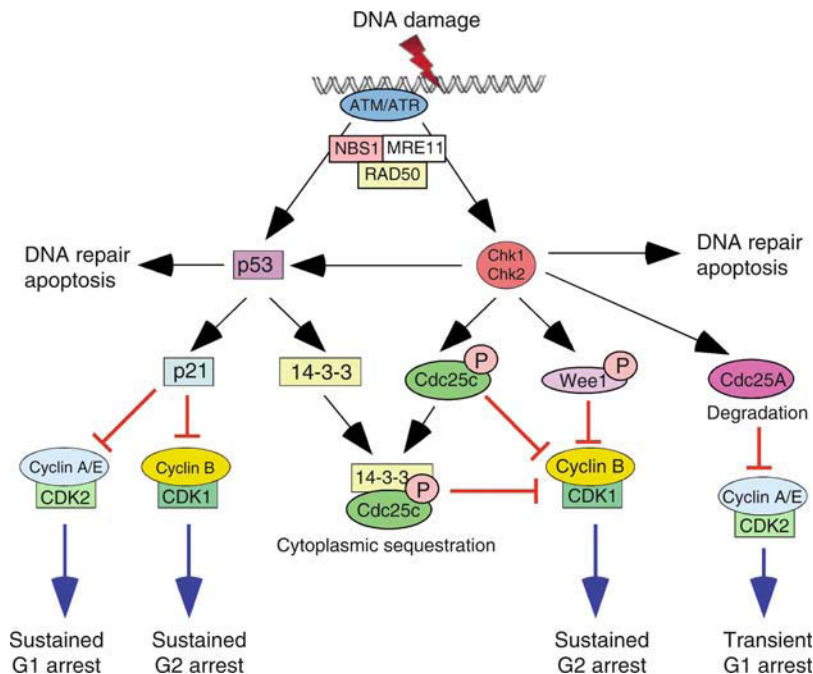
Cell Cycle Checkpoints

The survival of cells depends on their ability to transmit the genetic information properly onto two daughter cells. The faithful transmission depends on proper

DNA replication during S phase, the absence of DNA damage throughout the cell cycle, and the equal distribution of the chromatids during mitosis. Signal transduction pathways referred to as cell cycle checkpoints monitor these requirements. The best example investigated so far is the checkpoint pathway activated upon DNA damage known as the DNA damage checkpoint (Fig. 3) [4]. The DNA damage checkpoint is activated by an initial activation of the Atm or Atr kinases. The Atm kinase seems to be activated in response to DNA damage whereas Atr is primarily activated following arrest of DNA replication forks. How these kinases sense the cellular damage is still not understood, but the Mre11/Rad50/Nbs1 complex might contribute to this initial kinase activation. The alert signal is then transduced to the Chk1 and Chk2 protein kinases. Both, Atm/Atr and Chk1/Chk2 are able to phosphorylate the transcription factor p53 leading to its accumulation and activation. p53 induces a large-number of target genes including *p21Cip1*. The p21 protein accumulates and results in a sustained cell cycle arrest at both, the G1/S and G2/M transition by inactivating CDK2 and CDK1 kinases, respectively. p53 also induces the transcription of DNA repair genes and genes responsible for the induction of ►**apoptosis**. The latter ensures that a damaged cell will rather die than transmitting damaged DNA onto the next generation. Thus, p53 is a central regulator of the DNA damage response. Tumor cells frequently lack functional p53, which can directly contribute to tumorigenesis by promoting the accumulation of DNA damage.

The activation of Atm/Atr and Chk1/Chk2 kinases also results in a p53 independent, but transient G1/S cell cycle arrest by targeting the phosphatase Cdc25A that is required to activate CDK2–Cyclin A/E. Enhanced activity of Atm/Atr–Chk1/Chk2, induces the ubiquitin dependent degradation of Cdc25A, thus preventing CDK2 activation at G1/S. At the G2/M transition, activated Atm/Atr–Chk1/Chk2 phosphorylates the phosphatase Cdc25C leading to its degradation, cytoplasmic sequestration by 14-3-3 sigma proteins and enzymatic inhibition. This results in a sustained p53 independent G2/M arrest upon DNA damage (Fig. 3) [4]. In addition, other upstream regulators of CDK1–cyclin B like Polo-like kinase 1 (Plk1) or Aurora A seem to be part of a DNA damage response and contribute to the cell cycle arrest at G2/M independent of p53.

The mitotic ►**spindle assembly checkpoint** monitors the correct alignment of chromosomes during ►**mitosis**. The spindle checkpoint is activated in the early phases of a normal mitosis and in response to defects that prevent a proper chromosome alignment (e.g., upon drug-induced spindle damage). Progression into anaphase is inhibited as long as all chromosomes are properly attached to the mitotic spindle and aligned at the metaphase plate. This checkpoint involves the function of several checkpoint



Cell Cycle Control. Figure 3 The DNA damage checkpoint. In response to DNA damage cells activate p53 dependent and independent checkpoint pathways leading to cell cycle arrest at G1/S and G2/M allowing DNA repair. If the cellular damage cannot be repaired, cells can initiate apoptosis.

proteins including Mad1, Mad2, Bub1, BubR1, Bub3, Cenp-E that are recruited to kinetochores when the checkpoint is activated. However, the mechanisms leading to checkpoint activation are still not well understood. Spindle checkpoint activation results in an activation of the terminal effector Mad2, which is an inhibitor of the APC/C ubiquitin ligase. Therefore, activation of the spindle checkpoint inhibits the mitotic degradation of securin and cyclin B preventing anaphase onset and mitotic exit.

Pharmacological Intervention

Regulators of the cell cycle are frequently altered in human cancer often resulting in deregulated CDK activities. A promising anticancer therapy is therefore the inactivation of CDK activities using pharmacological CDK inhibitors that lead to a proliferative arrest of cells. Prolonged cell cycle arrest results in induction of ▶apoptosis and therefore eliminates tumor cells. In addition, other kinases or regulators of the cell cycle might be suitable targets for anticancer drugs [5].

Cell cycle ▶checkpoints also represent potential targets for anticancer drugs. Cells damaged by genotoxic agents or spindle poisons activate the appropriate checkpoint and halt the cell cycle until the damage is repaired. When these checkpoints are abrogated, cells enter mitosis or S phase prior to repair of the genotoxic damage, which promotes cell death. Drugs abrogating cell cycle checkpoints might therefore sensitize cells to chemotherapy [5].

Pharmacological CDK Inhibitors

The rationale behind CDK inhibition during anticancer treatment is to stop hyperactive cell cycles and to inhibit the activity of cyclins that are frequently overexpressed in human cancer.

Currently, many different CDK inhibitors are explored in clinical trials. After the first generation of drugs, which included the natural products flavopiridol, paullone, and indirubin, several purine and pyrimidine analogs are now under investigation. These second generation drugs include olomoucine, purvalanol B, E7070, R-roscovitine, and BMS-387032. Preclinical results show that all these drugs arrest (tumor) cells in G1, S, or G2 and a prolonged cell cycle arrest is followed by the induction of apoptosis. While most of the CDK inhibitors have reasonable toxicity profiles, they do not show a high specificity, which might decrease their tumor cell selectivity [5].

Pharmacological Cell Cycle Inhibitors

Many cell cycle regulatory kinases are overexpressed in human cancer and are therefore prime targets for chemotherapy to be targeted selectively in tumor cells. Several kinases that regulate the progression of mitosis are tested as anticancer drug targets. These kinases include the Aurora kinases and the Plk1. Currently, Aurora inhibitors (e.g., hesperadin, ZM447439, and VX-680) as well as Plk1 inhibitors (e.g., HMN-214) are undergoing clinical trials. However, all kinase inhibitors act by blocking the ATP binding pocket of

the kinases and are therefore expected to be not entirely specific [5].

Also nonkinase molecules might be important targets for anticancer drugs. For instance, the activity of the mitotic kinesin Eg5 (KSP, kinesin-5) can be inhibited by small molecules (e.g., monastrol, KSP-IA) resulting in mitotic defects associated with the induction of apoptosis.

Due to their promising activities in preclinical investigations, the first clinical data for these new generations of anticancer drugs are eagerly awaited.

Pharmacological Cell Cycle Checkpoint Abrogation

The introduction of DNA damage leads to the activation of the DNA damage checkpoint resulting in G1 and G2 cell cycle arrest. Overriding this arrest and inappropriate induction of S phase or mitosis, respectively, results in cell death in most cases. Thus, drugs that facilitate these transitions might sensitize cells to chemotherapy. Prime targets for checkpoint abrogating drugs are the Atm/Atr and the Chk1/Chk2 kinases. Atm can be inhibited by several methylxanthine derivatives (e.g., caffeine and pentoxifylline). However, the cytotoxicity of these drugs limits their use in patients.

In contrast, UCN-01, a staurosporine derivative, acts as a potent inhibitor of the Chk1 kinase and efficiently abrogates the G2 checkpoint upon DNA damage. The forced entry into mitosis in the presence of DNA damage results in a mitotic form of apoptosis. Several clinical trials are currently exploring a combined treatment with UCN-01 and various DNA damaging drugs. In the same vein, inhibitors of Chk2 are developed and tested in clinical trials.

In contrast to the DNA damage checkpoint, the mitotic spindle checkpoint is essential for cell viability. Therefore, targeting kinases of the spindle checkpoint including Bub1, BubR1, and Mps1 might be a valid strategy for anticancer treatment.

► Ubiquitin/Proteasome

► Apoptosis

► Cancer, Molecular Mechanisms of Therapy

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Cell Division Cycle

► Cell Cycle Control

Cell Multiplication

► Cell Cycle Control

Cell Proliferation

► Cell Cycle Control

Cellular Immunity

In a strict sense the term cellular immunity describes the T-lymphocyte mediated arm of adaptive immunity. T-lymphocytes recognize antigens in form of peptides presented by cells on major histocompatibility antigens (MHC molecules) indicating the phagocytosis of bacteria (presentation of antigen on MHC class II) or infection of a cell by a virus (presentation on MHC class I). T-lymphocytes become activated and clonally expand to become T-helper cells, which can induce an inflammatory response via soluble mediators (cytokines), which can activate the phagocytic system or induce specific antibody production. Alternatively, T-helper cells can activate cytotoxic T-cells, which kill virus infected cells or tumor cells.

► Immune Defense

Central Core Disease (CCD)

Central core disease (CCD) is an autosomal dominant, non-progressive myopathy characterized by hypotonia and proximal muscle weakness in infancy. CCD is named after detection of characteristic “central cores” that lack both mitochondria and oxidative enzyme

activity within type 1 muscle fibres biopsied from the patients. CCD may be associated with susceptibility to malignant hyperthermia. CCD is linked to a gene for type 1 ryanodine receptor (RyR1).

► Ryanodine Receptor

Central Diabetes Insipidus

In central diabetes insipidus a hypophysial malfunction, caused by different diseases as well as head injuries, neurosurgery, or genetic disorders, leads to AVP hyposecretion. This type of diabetes insipidus can successfully be treated by the exogenous administration of AVP or AVP analogues (e.g. desmopressin).

► Vasopressin/Oxytocin

Central Tolerance

In immunology, tolerance describes the absence of immune reactions against an antigen, mostly against antigens of the own body (self tolerance). Self tolerance can result from eliminating autoreactive lymphocytes when they develop in the central differentiating organs, thymus, or bone marrow (central tolerance), or from keeping lymphocytes, which escaped this central mechanisms into the periphery at rest by active suppressive mechanisms (peripheral tolerance).

► Immune Defense

► Tolerance and Desensitization

Centrosome

Centrosomes, also called the microtubule organizing centre, are protein complexes that contain two centrioles (ringlike structures) and γ - tubulin. They serve as nucleation points for microtubular polymerization and constrain the lattice structure of a microtubule to 13 protofilaments. They are responsible for organizing the mitotic spindle during mitosis.

► Cytoskeleton

► Cell Cycle Control

Cephalosporins

Like penicillins, cephalosporins are β -lactam antibiotics and interfere with bacterial cell wall synthesis. A very large number of cephalosporins are available for clinical use. They differ in their route of administration and clinical use.

► β -Lactam Antibiotics

CFTR

CFTR, the Cystic fibrosis transmembrane conductance regulator, is the only member of the large ABC transporter gene family that is known to function as an ion channel. Its “ATP Binding Cassettes” are thought to regulate the opening and closing of the pore, rather than provide energy (by ATP hydrolysis) for active transport. Another important mechanism of regulation is provided by cyclic AMP (cAMP)-dependent phosphorylation of residues in the intracellular “R-domain.” This renders CFTR into a cAMP-activated chloride channel.

CFTR has a single-channel conductance of about 8 pS. It is present in the apical membranes of many epithelia. Its mutation leads to the potentially lethal disease cystic fibrosis. In addition to acting as a chloride channel, CFTR is also thought to regulate, e.g., the epithelial sodium channel ENaC, a molecularly unknown outwardly-rectifying chloride channel, and possibly also potassium channels and water channels. Some of these potential regulatory processes, however, are controversial. CFTR also acts as a receptor for bacteria.

► Cl^- Channels

► Epithelial Na^+ Channels

cGMP

► Cyclic Guanosine Monophosphate

► Guanylyl Cyclase

► Nitric Oxide

► Carbon Monoxide

cGMP Kinase

cGMP-dependent protein kinase is encoded by two genes that encode for cGMP kinase I α and I β and cGMP kinase II. cGMP kinase I α and I β are isozymes that differ only at the amino terminus. The amino terminus controls the activity of the enzyme and interacts very specific with different targets.

- Nitric Oxide
- Guanylyl Cyclase
- Carbon Monoxide
- Smooth-Muscle-Tone Regulation
- Cyclic AMP- and Cyclic GMP-dependent Protein Kinases
- Adenylyl Cyclases
- Guanylyl Cyclase

cGMP-regulated Phosphodiesterases

Phosphodiesterases represent a multi-gene family of enzymes that hydrolyze the second messengers cGMP and cAMP. The hydrolytic activity of several sub-families of these enzymes is regulated in an allosteric manner by the binding of cGMP. The cyclic nucleotide binding site present in cGMP-regulated phosphodiesterases is not homologous to that found in most other cyclic nucleotide-binding proteins.

- Phosphodiesterases

CGRP

- Calcitonin Gene Related Peptide

Chagas Disease

Vector-born infectious disease caused by *Trypanosoma cruzi*.

- Antiprotozoal Drugs

Channelopathies

“Channelopathies” refer to a class of diseases caused by ion-channel dysfunction. Autoimmune, drug, toxic or genetic mechanisms underlie channelopathies. Mutations in genes encoding ion-channel proteins that alter channel function are common mechanisms underlying the cause of channelopathies. Examples of channelopathies include some forms of Bartter Syndrome (Kir1.1), Andersen’s Syndrome (Kir 2.1), Weaver Mouse Phenotype (Kir 3.2), Persistent Hyperinsulinemic Hypoglycemia of Infancy (PHHI) (Kir 6.2/SUR), Short QT Syndromes (Na and K channels, including Kir 2.1).

- K⁺ Channels
- Inwardly Rectifying K⁺ Channels
- ATP-dependent K⁺ Channels
- Voltage-dependent Ca²⁺ Channels
- Ryanodine Receptor
- Voltage-dependent Na²⁺ Channels

Chaperones

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Synonyms

Molecular chaperones, stress proteins (note: not all stress proteins are molecular chaperones and not all molecular chaperones are stress proteins); Heat shock proteins (Hsp); Polypeptide chain binding proteins

Definition

Chaperones bind to exposed hydrophobic surfaces of polypeptide substrates, and through either ATP-dependent or ATP-independent mechanisms facilitate the folding/assembly, intracellular transport, degradation, and activity of polypeptides.

Basic Mechanisms Molecular Chaperones

The term ►molecular chaperone was coined by Ron A. Laskey and coworkers in 1978 to describe the

properties of the nuclear protein nucleoplasmin in assisting the assembly of nucleosomes from isolated histones and DNA. Laskey introduced the term molecular chaperone to describe the function of nucleoplasmin because of the analogy with the human chaperone. The role of a human chaperone is to prevent incorrect interactions between pairs of human beings, without either providing a blueprint (or steric information) necessary for their correct interaction or the necessity to be present during their continued interaction. This concept was extended by Hugh R. B. Pelham in 1986. Pelham proposed that members of the Hsp70 and Hsp90 protein families are involved in the assembly and disassembly of proteins in the cytosol, nucleus, and **▶endoplasmic reticulum** (ER) under nonstress conditions, but are required in increased amounts during cellular stress (such as heat shock-conditions), both to unscramble protein aggregates and to prevent aggregation by binding to hydrophobic surfaces that are exposed as a result of the stress. In addition, Pelham

proposed that all cells contain a variety of proteins that act as molecular chaperones, in preventing incorrect interactions during the operation of several basic cellular processes under normal growth conditions. These cellular processes are protein folding and protein topogenesis in the course of protein biogenesis. Relatively recently, it was appreciated that certain molecular chaperones can also act as modulators/regulators of the activity of folded proteins. Furthermore, these proteins have also been shown to facilitate protein degradation, thereby making them important integration points of the cellular pathways of protein folding and degradation. Therefore, at a broad cellular level, molecular chaperones are some of the key quality control components of the cell.

Today, molecular chaperones are divided into three principle classes, according to their mode of action (Table 1). The one class comprises the chaperonins or Hsp100 protein family members. Chaperonins are oligomeric proteins, composed of two rings placed

Chaperones. Table 1 Molecular Chaperones in Humans (note: this list is not meant to be exhaustive). Synonyms are given in parentheses

Protein type	Cytosol	Endoplasmic reticulum	Mitochondria
sHsp	Hsp27 α -crystallin	–	–
Hsp70	Hsc70 Hsp70.1-3 Hsp70L1	Grp78 (BiP) Grp170	Grp75 (mtHsp75)
Hsp40	Hsp40 (DnaJB1) Hdj2 (DnaJA1) Auxilin (DnaJC6) P58IPK (DnaJC3) MPP11 (DnaJC21) CSP (DnaJC5) Hsj1 (DnaJB2)	ERj1 (DnaJC1) ERj2 (Sec63,DnaJC2) ERj3 (DnaJB11) ERj4 (DnaJB9) ERj5 (DnaJC10)	TID1 (DnaJA3) Tim14 (DnaJC19) Tim16 Tim44
NEF	Hspbp1 (Hsp110) Hspbp2	Grp170 Sil1 (BAP)	mtGrpE
Additional Co-chaperones of Hsp70	Hip Hop CHIP Bag1 TPR1 Tom70		
Hsp60	TRIC (CCT)	–	Cpn60 (mtHsp60)
Co-chaperones of Hsp60	Prefoldin (GimC)	–	Cpn10 (mtHsp10)
Hsp90	Hsp90	Grp94	–
Hsp100	Hsp100 (Skd3)	–	mtClpX (mtHsp100)
Other Chaperones	NAC SRP nucleoplasmin	Hsp47 Calreticulin Calnexin	NDUFAF1 B17.2L

back to back, each one enclosing a cavity that allows protein folding in a secluded environment. The term Anfinsen cage was coined by R. John Ellis in 1994 to illustrate this mechanistic principle. They are divided in two groups, those found in eubacteria or endosymbiotic organelles and those found in archaea and the eucaryotic cytosol. The second class includes many of the other Hsp protein families (sHsp, Hsp70, Hsp90, Hsp100) and their respective **co-chaperones** (such as Hsp40 and nucleotide exchange factors, NEF). These chaperones have highly diverse three-dimensional structures but share as a mechanistic principle that they aid protein folding and protein transport by cycles of binding and release of exposed hydrophobic surfaces of polypeptides that, otherwise, would be prone to aggregation. This mode of action is described in detail below in the case of Hsp70. The third class represents polypeptide binding proteins that appear to be more specialized for certain types of substrate polypeptides, such as nascent polypeptide chains (NAC, nascent polypeptide associated complex; SRP, signal recognition particle) or glycoproteins, (Calnexin, Calreticulin) or even single substrate proteins (nucleoplasmin, Hsp47, NDUFAF1, B17.2L).

The Hsp70 chaperone system is the most widely distributed chaperone system in the human cell (Fig. 1). The major molecular chaperone, Hsp70, consists of an N-terminal ATPase domain and a C-terminal substrate binding domain. The affinity of Hsp70 for protein substrates is modulated by ATP binding and hydrolysis. In the ATP bound state, affinity of the substrate binding domain for the substrate protein is low and exchange rates are high. Hydrolysis of ATP to ADP results in high affinity for the substrate and low exchange rates, effectively locking the substrate into the binding pocket. This integral step in assisted protein folding is directly modulated by the binding of Hsp40 proteins. Hsp40s are defined by the presence of an approximately 70 amino acid region known as the J-domain, which is essential for interaction with Hsp70. The J-domain is a highly conserved α -helical structure that interacts with

the Hsp70 ATPase domain and possibly also with the Hsp70 substrate binding domain. Hsp40s are divided into three groups based on their possession of domains in addition to the J-domain. Type I Hsp40s contain four domains: an N-terminal J-domain, a glycine/phenylalanine (GF)-rich region, a zinc finger domain and a C-terminal domain. Type I Hsp40s have been shown to bind protein substrates at their C-terminal domain and to have independent chaperone activity by inhibiting denaturation and aggregation. Type II Hsp40s contain an N-terminal J-domain, a GF-rich region and a C-terminal domain. Type III Hsp40s contain the J-domain, and this may occur at any position within the protein. Other than the J-domain, the type III Hsp40s are highly divergent in size, sequence and structure and tend to serve highly specialized functions.

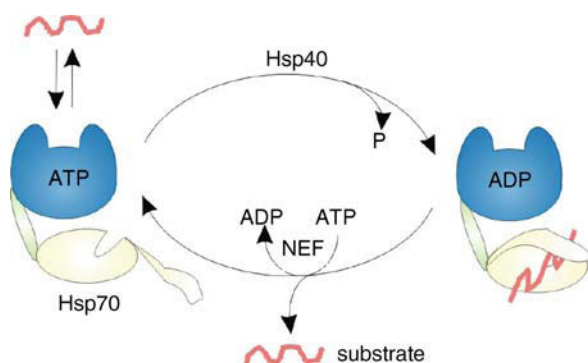
The various roles of a certain Hsp70 shall be described with respect to Grp78 (also called immunoglobulin heavy-chain binding protein, BiP) (Fig. 2). BiP is the Hsp70 protein family member resident in the ER. It is involved (i) in the import of polypeptides into the ER – working as a structural modulator of the folded Sec61 complex as well as a molecular ratchet on the incoming polypeptide-, (ii) in folding and assembly of proteins in the ER, (iii) in the export of misfolded polypeptides for degradation by the proteasome (**ERAD**), and (iv) in the so-called unfolded protein response (**UPR**).

RNA Chaperones

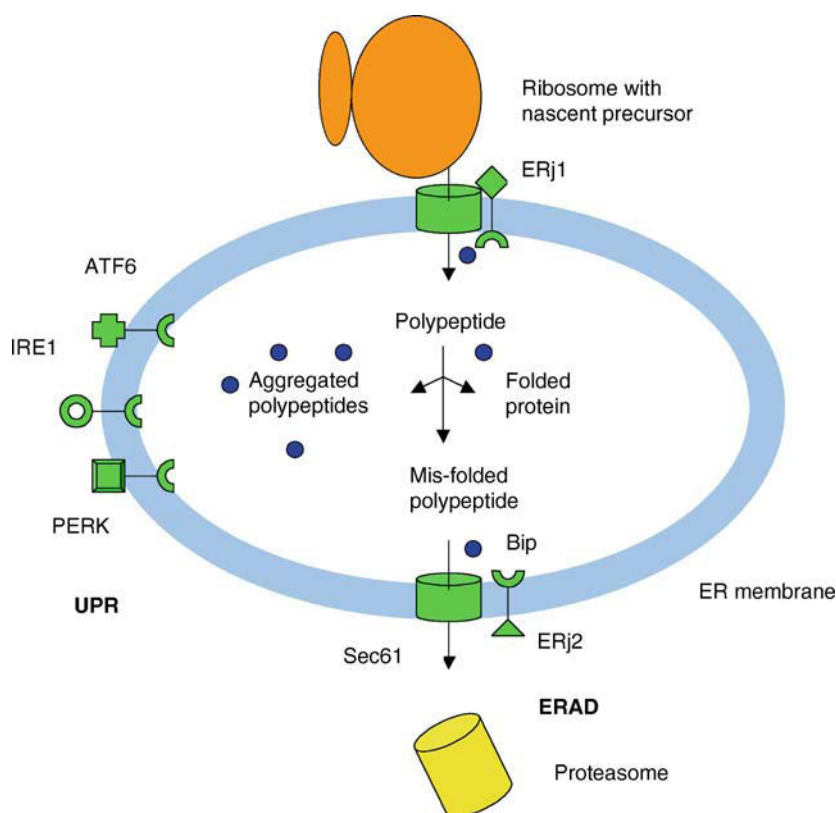
As it is the case in polypeptide folding, nonspecific or promiscuous RNA-binding proteins can prevent RNA mis-folding and resolve mis-folded RNAs, thereby ensuring that RNA is accessible for its biological function [1]. Certain DEAD-box proteins as well as some proteins that are involved in the assembly of ribonucleoparticles were shown to act as RNA chaperones.

Chaperone Diseases

There are a number of human diseases associated with mutations in genes encoding chaperones, and the functional failure of certain molecular chaperones has been linked to human diseases [2]. In addition, disease-linked mutations have been found in proteins that regulate the activity of chaperones (i.e., co-chaperones). To name just a few examples and to stay with the Hsp70 chaperone network in the ER (Fig. 2) Marinesco–Sjögren syndrome (MSS; OMIM 248800) and one form of polycystic liver disease (PCLD; OMIM 174050) have been linked to Sil1 and ERj2 (Sec63), a nucleotide exchange factor and Hsp40, respectively, for BiP [3]. In addition, over-expression of various molecular chaperones as well as mutations in certain chaperone genes has been observed in a wide range of tumors [3]. In some cases, the chaperone over-expression was linked to poor prognosis and/or



Chaperones. Figure 1 The functional cycle of Hsp70



Chaperones. Figure 2 The multiple roles of BiP in the biogenesis of the secretory proteins. BiP, immunoglobulin heavy chain binding protein; ER, endoplasmic reticulum; ERAD, ER-associated degradation; ERj, resident ER protein with J-domain; Sec61, core subunit of the protein translocator; UPR, unfolded protein response that involves several signal transduction pathways that are activated in order to increase the biosynthetic capacity and decrease the biosynthetic burden of the ER

resistance to therapy. Furthermore, recent work linked a human infectious disease to inactivation of the ER-resident chaperone BiP [3]. The AB₅ subtilase cytotoxin of certain pathogenic bacteria causes morbidity and mortality among children in developing countries by inactivating BiP.

Pharmacological Intervention

Chemical Chaperones

A number of different low molecular weight compounds are known to stabilize proteins in their native conformation and, therefore, may be effective in correcting of protein folding abnormalities *in vivo*. Relevant compounds are *N*-acetyl-L-lysine, L-carnitine, taurine, betaine, ectoine, and hydroxy-ectoine [4]. Some of these ►[chemical chaperones](#) and ►[pharmacological chaperones](#) are already used in clinical trials to combat protein folding diseases, such as cystic fibrosis.

Drugs that Target Molecular Chaperones

Several small compounds have been discovered that alter expression or function of Hsp70 and Hsp90,

respectively [5]. The proteasome inhibitor Bortezomib (Velcade[®]) leads to accumulation of mis-folded proteins and, therefore, stress response and subsequent induction of stress proteins, such as Hsp70; the natural compound celastrol as well as geranylgeranylacetone (GGA) were also shown to induce a stress response. Thus, these compounds may some day be used to combat protein folding diseases. Furthermore, small molecules with structural similarity to 15-deoxyspergualin (DSG) or NSC 630668.R/1 were described to inhibit the ATPase activity of Hsp70. Thus, these compounds may be useful in combating certain cancers. Furthermore, based on the discovery of the Hsp70-inhibitors, screening for positive modulators is pursued. The established anti-cancer drug geldanamycin and its less toxic analogue 17-AAG (17-allylaminogeldanamycin), on the other hand, target Hsp90.

►Protein Trafficking and Quality Control

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ChE

► Cholinesterases

Cheese Reaction

Hypertensive reaction resulting from release of noradrenaline by tyramine and other sympathomimetic amine as a consequence of irreversible inhibition of MAO-A.

► Monoamine Oxidases and their Inhibitors

Chemical Chaperone

A small compound that mimicks a molecular chaperone in activity.

► Chaperones
► Protein Trafficking and Quality Control

Chemical Library

A chemical library is a precisely defined collection of different chemical compounds. Chemical libraries can be either prepared by parallel synthesis or by split-and-recombine synthesis.

► Combinatorial Chemistry

Chemical Neurotransmission

Chemical neurotransmission is the way in which neurons communicate by releasing chemical substances that are received by the receptors in the next neuron (or the target) and excite or inhibit it. About 50% or more of drug mechanisms are based on modification of chemical neurotransmission.

► Synaptic Transmission

Chemoattractant Receptors

► Chemokine Receptors

Chemokine Receptors

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Synonyms

Chemoattractant receptors; Chemokine receptors

Definition

► **G-protein-coupled receptors** (GPCRs) play a major role in regulating the overall homeostasis of complex organisms, like mammals, but are also found in primitive species like *Dictyostelium* (slime mold) and yeast [1]. The GPCR superfamily is quite diverse and sequencing has revealed more than 850 genes comprising around 3% of the human genome. The diversity of the GPCRs is equally matched by the variety of ligands that activate them, which include odorants, taste ligands, light, metals, biogenic amines, fatty acids, amino acids, peptides, proteins, nucleotides, lipids, Krebs cycle intermediates, and steroids. Because of their central role in regulating normal physiological responses, GPCRs have attracted considerable attention from the pharmaceutical industry as targets for disease. This large superfamily of proteins remains one of the most druggable targets accounting for more than 40% of all marketed therapeutics. Chemokine receptors are members of the GPCR superfamily and seven CXC, ten

CC, and one CX3C and XC chemokine receptors have been cloned so far [2]. Receptor binding initiates a cascade of intracellular events mediated by the receptor-associated heterotrimeric G-proteins. These G-protein subunits trigger various effector enzymes that lead to the activation not only of ▶chemotaxis but also to a wide range of functions in different leukocytes such as an increase in the respiratory burst, degranulation, ▶phagocytosis, and lipid mediator synthesis.

Basic Characteristics

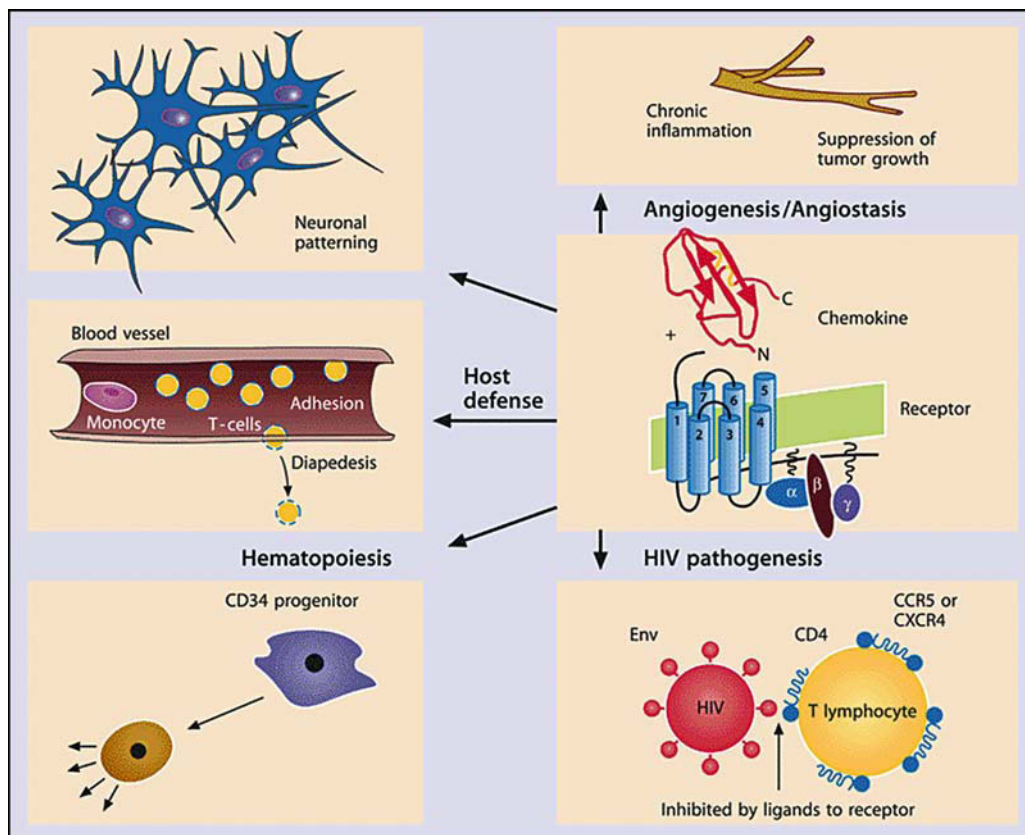
Chemokines belong to a large family of small, chemotactic cytokines characterized by a distinctive pattern of four conserved cysteine residues [3]. They are divided into two major (CXC and CC) and two minor (C and CX3C) groups dependent on the number and spacing of the first two conserved cysteine residues. Although originally identified on the basis of their ability to regulate the trafficking of immune cells, the biological role of chemokines goes well beyond this simple description of their function as chemoattractants; they have been shown to be involved in a number of biological processes, including growth regulation, hematopoiesis, embryologic development, angiogenesis, and ▶HIV-1 infection (Fig. 1).

Chemokines have been shown to be associated with a number of autoimmune diseases including ▶multiple sclerosis, ▶rheumatoid arthritis, atherosclerosis, dermatitis, and organ transplant rejection. Evidence, reviewed below, is mounting that chemokines may play a major role in the pathophysiology of these diseases and thus chemokine receptor ▶antagonists could prove to be useful therapeutics in treating these and other proinflammatory diseases.

Chemokine Receptors

Although leukocytes continue to be the major site of expression of chemokine receptors, several studies have recently demonstrated chemokine receptor expression on neurons in the CNS.

A number of chemokine receptors including CXCR2, CXCR4, CCR1, CCR5, and DARC have been demonstrated in either adult or fetal brain. Not only were these receptors present on the cell surface but they were also functional. Clearly, the role of these receptors on CNS neurons must be very different from their role on immune cells. Given that human astrocytes can be stimulated with cytokines to upregulate the expression of chemokines, it is tempting to speculate that in vivo during CNS development, chemokines



Chemokine Receptors. Figure 1 Biological functions of chemokine receptors as exemplified by the chemokine receptor CXCR4.

secreted by astrocytes might engage specific receptors expressed on neurons and may play a role in the directed migration of specific subsets of neurons to distinctive regions of the brain.

Genetic mutations of receptors, both natural and induced (by targeted gene disruption), can help to unravel their biological roles. Nature has been generous in this regard by providing us with two naturally occurring examples of gene inactivation for chemokine receptors. Humans homozygous for inherited inactivating mutations of the Duffy (*DARC*) gene and the *CCR5* gene have been identified and appear to be phenotypically normal and healthy. Indeed, these gene inactivations appear to be beneficial to their hosts, rendering them resistant to certain infectious diseases. For example, DARC-negative individuals are resistant to malaria induced by *Plasmodium vivax*, which utilizes DARC to attach to and enter erythrocytes. CCR5-negative individuals are resistant to HIV-1, which utilizes this chemokine receptor as a coreceptor for invasion (see section role of receptors in HIV infection).

Analysis of receptor-inactivated individuals can also be useful in clarifying the role of these receptors in disease. For example, it is known that CCL3 appears to play an important role in multiple sclerosis. This chemokine is a potent ▶agonist for both CCR1 and CCR5 receptors, opening the possibility that either of these receptors could be involved in mediating the development of the pathophysiological changes seen in this disease. However, analysis of a large group of individuals, comprising both normal subjects and those suffering from relapsing/remitting multiple sclerosis, showed that there was no significant difference in the allele frequency of the CCR5 mutation between the groups. These studies indicate that CCR5 is not an essential component in the expression of multiple sclerosis and implicates CCR1 in the disease.

Role in Immune Response

Chemokines are potent chemoattractants that provide directional cues to summon leukocytes. Leukocyte recruitment is a three-step process that involves the formation of solid phase chemokine gradients generated by the binding of chemokines to extracellular matrix proteins like glycosaminoglycans, which decorate the cell surface of endothelial cells. These gradients then attract immune cells that first undergo selectin-mediated rolling along the endothelial cells. Chemokine-mediated upregulation of CD11/18 complexes then results in a much firmer adherence of immune cells to the endothelium and this culminates in diapedesis of leukocytes across the endothelial space into tissues.

By regulating the movement of different subsets of leukocytes from the peripheral blood to extravascular sites such as organs, skin, or connective tissue,

chemokines play a critical role in the maintenance of host defence as well as in the development of the immune response. However, sometimes these molecules can inappropriately target immune cells to attack their own tissues and organs leading to inflammation and cellular destruction. Indeed, strong evidence supports the idea that chemokines play an important role in the pathogenesis of a number of autoimmune diseases such as multiple sclerosis and rheumatoid arthritis.

Rheumatoid arthritis is a chronic inflammatory disease characterized in part by a memory T lymphocyte and monocyte infiltrate. The interaction of the same cell types also play a major role in the demyelinating processes that culminate in multiple sclerosis. Recent studies using neutralizing antibodies have provided strong in vivo concept validation for a role of chemokines in animal models of both diseases. For example, in an adjuvant-induced arthritis (AIA) model in the rat, antibodies to CCL5 were able to abrogate the development of the disease by greatly reducing the infiltration of mononuclear cells into tissue joints. Similarly, antibodies to CCL3 prevented the development of both initial and relapsing paralytic disease as well as infiltration of mononuclear cells into the central nervous system of a mouse experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis. These results strongly suggest that chemokines play important roles in T-cell-mediated autoimmune diseases.

In contrast to the role of T-cells and monocytes in chronic inflammation, the primary hallmark of acute inflammatory diseases, such as empyema, acute lung injury, acute respiratory distress syndrome (ARDS), and dermatitis, is tissue infiltration by neutrophils. Thus, neutrophil-activating CXC chemokines-like CXCL8 are most often associated with these diseases. Clear evidence for a role of CXCL8 in acute lung injury and pleurisy has been provided by the finding that antibodies to CXCL8 dramatically increases the survival time of rabbits in models of disease. Not only did the antibodies increase the alveolar–arterial oxygen difference of the animals, increasing the oxygenation of the blood and therefore decreasing breathing difficulties, but they also affected a significant reduction in the infiltration of neutrophils into the lung.

Based on the demonstrated role of chemokines in disease, the generation of small molecule chemokine receptor antagonists have received great interest from pharmaceutical companies as attractive therapeutic approaches. GPCR's like chemokine receptors have in the past been an extremely fertile source of biological targets in the pharmaceutical industry, and compound library screening has proven successful in the discovery of antagonists for a number of these receptors, i.e., CCK and neurotensin antagonists. Using similar approaches

several drug companies have now identified potent small molecule antagonists of a number of chemokine receptors, which should find broad utility in a variety of acute and chronic inflammatory diseases.

Drugs

Insight into the physiological and pathophysiological roles of chemokine receptors have been provided by studies with potent receptor antagonists for CCR1, CCR2, CCR3, CCR4, CCR5, CCR9, CXCR2, CXCR3, and CXCR4, reviewed in Ribeiro and Horuk [4]. A number of these antagonists are in human clinical trials (Table 1). For example, CCR5 antagonists are in phase III clinical trials for the treatment of AIDS and CCR9 antagonists are in phase II clinical trials for inflammatory bowel disease.

Whether these antagonists live up to their initial promise as potent therapeutics for autoimmune disease or not is still open to question. With the exception of the CCR5 antagonist whose target is a viral disease, AIDS, the other antagonists are being used to treat complex human diseases. Just recently three of these clinical trials were reported as failures [4]. The first of these was the Pfizer CCR1 antagonist, CP-481715, which entered Phase II trials in February 2004 for rheumatoid arthritis. Although the compound was well tolerated, data suggested that it did not exhibit efficacy in rheumatoid arthritis patients and the trial was stopped following 6 weeks of treatment. The Berlex/Schering AG oral CCR1 antagonist BX 471 entered phase II clinical trials for multiple sclerosis in early 2004. Again the drug was well-tolerated and showed no safety concerns but its development was stopped after the clinical Phase II study failed to show a reduction in the number of new inflammatory CNS lesions, as detected by Magnetic Resonance Imaging. Finally, a CXCR3 antagonist from Amgen recently entered a Phase II trial

for psoriasis, unfortunately the inhibitor failed to demonstrate any signs of efficacy and the trial was terminated.

What do these failures tell us? These clinical trials could have failed for a variety of reasons for example was sufficient drug on board, was the right target selected, how reliable were the animal efficacy models that preceded the clinical trial, what markers if any were utilized in order that the correct patient population that could respond to the drug was selected? Since we do not know the answers to all of these questions it is difficult to ascribe a reason for the failure of these approaches. One idea that we can however propose is that perhaps targeting complex heterogeneous diseases with a drug that targets a single receptor is not the right approach. Roth et al. have for example advocated a “magic shotgun” rather than a “magic bullet” approach to target complex diseases such as cancer and schizophrenia [5]. In line with this suggestion it is interesting that one of the most successful drugs to target multiple sclerosis recently is the molecule FTY720 which targets four out of the five GPCRs that are activated by sphingosine 1-phosphate. FTY720 is currently entering phase III clinical trials and the phase II data look exciting. Based on the failures of drugs that target a single chemokine receptor (magic bullet) perhaps promiscuous molecules, like FTY720, that target several chemokine receptors (magic shotgun) will be more successful. This is certainly possible for example CCR5 antagonists like TAK 779 and TAK 652 also bind with high affinity to CCR2 and the CCR3 antagonist UCB35625 also binds with high affinity to CCR1.

Chemokine Receptors in Pathogen Infection

A number of viruses, including those in the Herpes and Pox families, express chemokine-like or chemokine receptor-like molecules that presumably help them to

Chemokine Receptors. Table 1 Chemokine receptor antagonists in clinical trial

Receptor	Company	Clinical phase	Compound	Indication	Status
CCR1	Berlex	II	BX 471	MS	Failed in MS
CCR1	Pfizer	II	CP-481715	RA	Failed in RA
CCR1	Millennium	I	MLN 3897	RA, MS	
CCR2	GSK	I		MS	
CCR3	GSK	I	766994	Asthma and allergic rhinitis	
CCR3	BMS	I	DPC-168	Asthma	
CCR5	Pfizer	III	UK-427857	HIV	
CCR5	Schering	II	SCH-C	HIV	
	Plough		SCH-D		
CCR9	Chemocentryx	II	Traficet-EN®	IBD	
CXCR3	Tularik	II	T487	MS Psoriasis	Failed in Psoriasis
CXCR4	Anormed	III	AMD3100	Stem cell transplant	

survive immune attack and proliferate. In addition to these strategies, chemoattractant receptors have themselves been targeted as vehicles of cellular invasion by a wide variety of microbes. These range from the Duffy blood group antigen, a promiscuous chemokine receptor on human erythrocytes which serves as a binding protein for the malarial parasite *Plasmodium vivax*, to the fractalkine receptor, CX3CR1, which is a portal of entry for the respiratory syncytial virus, and the HIV-1 virus which utilizes the chemokine receptors CXCR4 and CCR5 as coreceptors for cellular entry. CCR5 is an entry cofactor for M-tropic isolates of HIV-1 and is important in the early proliferative part of the disease, while CXCR4 is a coreceptor for T-tropic isolates of HIV-1 whose emergence in infected individuals usually correlates with accelerated disease progression.

HIV-1 resistance exhibited by some exposed but uninfected individuals is due, in part, to a 32 base pair deletion in the *CCR5* gene (*CCR5D32*) which results in a truncated protein that is not expressed on the cell surface. About 1% of Caucasians are homozygous for the *CCR5D32* allele and appear to be healthy with no untoward signs of disease. In fact, recent findings suggest that homozygosity for the *CCR5D32* alleles confers other selective advantages to these individuals, rendering them less susceptible to rheumatoid arthritis and asthma and prolonging survival of transplanted solid organs.

- Cytokines
- Immune Defense

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Chemokines

Chemokines are a family of small cytokines, or proteins secreted by cells. Proteins are classified as chemokines according to shared structural characteristics such as small size (8–10 kDa in size), and the presence of four

cysteine residues in conserved locations that are key to forming their three-dimensional shape. Chemokines have been identified as attractants of different types of blood leukocytes to sites of infection and inflammation. They are produced locally in the affected tissues and act on leukocytes through selective chemokine receptors. Chemokines help to control leukocyte maturation, traffic, and homing of lymphocytes and the development of lymphoid tissues.

- Chemokine Receptors
- Inflammation
- Immune Defense
- Matrix Metalloproteinases
- Cytokines

Chemoreceptor Trigger Zone

The chemoreceptor trigger zone (CTZ) is a group of neurons in the area postrema of the medulla. Once stimulated, it activates the vomiting center, which is also located in the medulla, thereby causing emesis. The CTZ is sensitive to a variety of chemical stimuli. Syrup of ipecac (synonym ipecacuanha) and apomorphine are direct stimulators of the CTZ. Clinically they are used to provoke emesis after oral ingestion of a poison. The CTZ is also stimulated by other drugs, e.g. cardiac glycosides, morphine (opioid system) and antineoplastic agents.

- Emesis

Chemotaxis

Chemotaxis is the detection of and coordinated movement toward a chemical compound by a cell or organism, e.g., neutrophils move towards an area of infection because of chemicals released by infected tissues.

- Chemokine Receptors
- Hematopoietic Growth Factors
- G-protein-coupled Receptors
- Cytokines
- Immune Defense
- Phosphodiesterases

Chemotherapy

Treatment for tumor patients with synthetic drugs – chemotherapeutics – that may be of completely different chemical structure. The main goal of tumor chemotherapy is to achieve a selective toxicity for the tumor without causing damage to the host, for instance by combining several cytostatic drugs at doses lower than required for monotherapy.

- ▶ Cancer, Molecular Mechanisms of Therapy
- ▶ Antineoplastic Agents
- ▶ Alkylating Agents
- ▶ Antimetabolites

Cholecystokinin

Cholecystokinin (CCK) is produced in the intestine and the brain. It appears to be an important mediator of anxiety. It also stimulates vasopressin secretion and slows gastric emptying. In addition, it is an important humoral satiety signal (appetite control). Various antagonists have been developed and are currently being investigated with regard to their therapeutic potential.

- ▶ Appetite Control
- ▶ Anti-obesity Drugs

Cholegraphic Contrast Agents

Cholegraphic contrast agents are ionic (acidic) iodinated molecules, which reversibly bind to albumen and are actively excreted into the bile.

- ▶ X-ray Contrast Agents and Molecular Imaging

Cholera Toxin

Cholera toxin is a protein toxin of *Vibrio cholerae*. Toxin ADP-ribosylates the α -subunit of the G_s heterotrimeric

GTP-binding protein at an arginine residue which is involved in GTP hydrolysis. ADP-ribosylation thus leads to constitutive activation of G_s .

- ▶ Heterotrimeric GTP-binding Proteins
- ▶ Small GTPases
- ▶ Bacterial Toxins

Cholesterol

Cholesterol is a widely distributed sterol found free or esterified to fatty acids. It is an important intermediate in the biosynthesis of steroid hormones and the principal component of cell plasma membranes and the membranes of intracellular organelles.

- ▶ HMG-CoA-Reductase Inhibitors
- ▶ Sterol Transporters
- ▶ Lipoprotein Metabolism

Cholesteryl Ester Transfer Protein (CETP)

Protein that transfers lipids among lipoproteins, especially cholesteryl ester from HDL to VLDL in exchange for triglycerides.

- ▶ Lipoprotein Metabolism

Cholinergic Transmission

Cholinergic Transmission is the process of synaptic transmission which uses mainly acetylcholine as a transmitter. Cholinergic transmission is found widely in the peripheral and central nervous system, where acetylcholine acts on nicotinic and muscarinic receptors.

- ▶ Cholinesterases
- ▶ Nicotinic Receptor
- ▶ Muscarinic Receptor
- ▶ Synaptic Transmission

Cholinesterase

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Synonyms

Acetylcholinesterase (EC 3.1.1.7) (AChE): Acetylcholine acetylhydrolase; True ChE; ChE I; ChE; Acetylthiocholinesterase; Acetylcholine hydrolase; Acetyl β -methylcholinesterase; Erythrocyte ChE

Butyrylcholinesterase (EC 3.1.1.8) (BChE or BuChE): ChE; Pseudocholinesterase; Plasma ChE; Acylcholine acylhydrolase; Non-specific ChE; ChEII; Benzoylcholinesterase; Propionylcholinesterase

Definition

Cholinesterases (ChEs), polymorphic carboxylesterases of broad substrate specificity, terminate neurotransmission at cholinergic synapses and neuromuscular junctions (NMJs). Being sensitive to inhibition by organophosphate (OP) poisons, ChEs belong to the serine hydrolases (B type). ChEs share 65% amino acid sequence homology and have similar molecular forms and active centre structures [1]. Substrate and inhibitor specificities classify ChEs into two subtypes:

1. Acetylcholinesterase (EC 3.1.1.7) (AChE)
Primarily hydrolyses esters with short acyl moiety, such as acetylcholine (ACh). It is the major ChE in human blood, muscle and brain cells. AChE mRNA is 20-fold more abundant than BChE mRNA.
2. Butyrylcholinesterase (EC 3.1.1.8) (BChE or BuChE)
Primarily hydrolyses esters with longer aliphatic (compared to AChE) or aromatic acyl moiety, such as butyrylcholine (BCh) and benzoylcholine (BzCh). BChE is the primary circulating ChE. It is threefold more abundant than AChE in human blood and is found in liver, lungs, muscles, brain and heart.

Basic Characteristics

Cellular Processes

ChEs control the duration of ACh-mediated action on post-synaptic receptors in cholinergic synapses, and have non-hydrolytic roles in nervous systems' development and plasticity.

Catalytic Activity

ChEs hydrolyze choline esters and carboxylic esters (1, 2 respectively):

1. $\text{RCO}_2(\text{CH}_2)_2\text{N}^+(\text{CH}_3)_3 + \text{H}_2\text{O} \rightarrow \text{RCO}_2\text{H} + \text{HO}(\text{CH}_2)_2\text{N}^+(\text{CH}_3)_3$
(R = CH₃ ACh; C₃H₇ BCh; C₆H₅ BzCh)
2. $\text{RCO}_2\text{R}' + \text{H}_2\text{O} \rightarrow \text{RCO}_2\text{H} + \text{HOR}'$

Hydrolysis involves a Glu-His-Ser catalytic triad, similar to other serine hydrolases, through a “charge relay” process. The imidazol ring of His relays electrons from Glu to Ser and the hydroxyl oxygen of Ser becomes a nucleophil and attacks the ester bond of a substrates, yielding an acyl-enzyme intermediate releasing a choline moiety. A water molecule then hydrolyzes the acetylated Ser by nucleophilic attack. Carbamates and OPs chemically modify the active site Ser, slowing down the last hydrolysis step and irreversibly inhibiting hydrolysis.

Cholinergic neurotransmission: ChEs terminate cholinergic transmission in the central nervous system (CNS), in NMJs and in the autonomic system (the parasympathetic system, somatic motor nerves and pre-ganglionic sympathetic nerves). A few sensory cells and the NMJ in nematodes also include ChEs.

Physiological roles: Both enzymes can catalyze ACh hydrolysis into acetic acid and choline, thus maintaining proper transmission of impulses between nerve cells or from neurons to muscles and gland cells. The catalytic mechanism of AChE is extremely efficient, approaching diffusion-controlled rates ($\text{kcat/Km} = 1.47 \times 10^8 \text{ M}^{-1}\cdot\text{s}^{-1}$), considerably faster than BChE's hydrolysis rate of ACh. BChE deficiency, an uncommon genetic disorder, causes no pathology, but increases carriers' susceptibility to prolonged apnea under muscle relaxant agents, such as succinylcholine or mivacurium, which are naturally destroyed by BChE. When administered under anaesthesia to a susceptible person, these compounds are ineffectively removed, which prolongs muscle relaxation. Likewise, subjects with BChE deficiency are susceptible for adverse reactions to other anti-ChEs.

Both ChEs show arylacylamidase activity, but BChE has a wider range of substrates: it hydrolyzes several choline esters from acetyl to heptanoylcholine, as well as other aliphatic esters. High BChE activity in many first contact tissues (e.g., lungs, skin, blood and placenta) as well as high affinity towards a wide range of orally ingested toxic compounds and xenobiotics (e.g., cocaine, procainamide, acetylsalicylic acid) support the hypothesis that BChE functions as a natural bioscavenger by absorbing and degrading OP poisons (e.g., nerve agents) before these cause neurological damage.

Non-Catalytic Activity

ChEs exist throughout the entire animal kingdom and appear in locations, where no ACh is released, suggesting that these possess additional non-hydrolytic functional properties, designated “non-classical”. These may depend on protein–protein interactions,

compatible with ChE's structural homology with synaptic proteins such as neuroligins, which interact with other synaptic proteins – the neurexins. Also, AChE contains two helix-loop-helix motifs, each forming a calcium-binding site (EF-hand). These do not play a role in its catalytic activity but may participate in protein–protein interactions.

Within the nervous system, ChEs were shown to be involved in membrane conductance and transmission of excitatory amino acids, learning and memory, neurite growth, neuritic translocation and acute stress reactions. Recent findings propose AChE's involvement in apoptosome formation [2].

Reports of ChE abnormalities in tumours, e.g., meningioma, glioma, acoustic neurinomas and lung cancers, megakaryocytopoietic disorders and leukemias, ovarian tumours and neuroblastomas, suggest cell proliferation and differentiation activities.

Structure

ChEs possess the α/β -fold structure, which is shared with other esterases and non-catalytic proteins such as thyroglobulin, glutactin, neurotactin, gliotactin and neuroligins, all of these include a single ChE domain. Both ChEs are ellipsoidal molecules of $\sim 45\text{--}60\text{--}65\text{ \AA}^3$. Their structure consists of a central, highly twisted, 8–12-stranded β -sheet, in which most strands are parallel, flanked on both sides by α -helices. Studies have indicated three major domains within the protein:

The active site is composed of two subsites:

The esteratic subsite contains the catalytic machinery of the enzyme. The catalytic triad residues – Ser 200, His 440 and Glu 327 (the residue numbering in this section refers to *Torpedo californica* acetylcholinesterase, *TcAChE*) – are identical in both enzymes and basically in the same positions.

The anionic subsite (Trp 84 and Phe 330) lies between the peripheral and acylation sites, half-way down the gorge and accommodates the positively charged quaternary ammonium of the choline moiety. Trp 84 orients the charged part of the substrate to the active centre. This subsite is involved in a “cross-talk” mechanism with the peripheral anionic site (PAS) [3].

The aromatic gorge composed of 14 highly conserved aromatic residues, is placed $\sim 20\text{ \AA}$ deep, penetrating half-way into the gorge harbouring the active site. In BChE, 6 of the 14 aromatic amino acid residues are replaced by aliphatic ones. Therefore, BChE's gorge is larger in volume and shows a distinct inhibitor sensitivity profile. Other crucial residue changes exist in the acyl-binding pocket, with the replacements of Phe

288 and Phe 290 of *TcAChE* by Leu 286 and Val 288, respectively; these changes enable the binding of the bulkier butyrate substrate moiety in BChE.

The PAS is located at the rim of the aromatic gorge, on the protein's surface. It spans six AChE residues: Tyr 72, Tyr 124, Glu 285 and Trp 286, on one side of the gorge entrance, and Asp 74 and Tyr 341, on its opposite side. Its core is comprised of Trp 286 and Asp 74, which accommodates many distinct ligands. BChE also has a PAS, but its relatively aromatic content and the response upon ligand-binding differ significantly from those of AChE.

Molecular Mechanisms of Assembly

ChEs present a wide molecular diversity that modulates their function in cholinergic synapses and non-synaptic contexts. This diversity arises at the genetic, post-transcriptional and post-translational levels.

Genetics and Evolution

Genomic comparisons suggest evolutionary gene duplications creating new ChE encoding genes, which initiated before the divergence of nematodes and insects. Subsequent independent duplications lead, for example, to four *ACHE* genes in nematodes. Some families or species may in turn have lost copies of the duplicated genes (e.g. *Drosophila melanogaster*). The duplication that formed *ACHE* and *BCHE* occurred in the tetrapod lineage, thus, amphibians and reptiles but not fish have a *BCHE* homologue resembling *BCHE* of mammals or birds.

The human ACHE gene (7q22) is 7 kb long, encodes all variants of AChE, which share a core domain, spanning the 543 amino acids encoded by exons E2, E3 and E4 of this gene (E1 is a non-coding exon). Post-transcriptional events modify the N'- and the C'-terminus, causing differences in assembly and localization of the AChE multiple forms. A single nucleotide polymorphism (SNP) in position 353 (His353Asn) is responsible for the Yt blood group system; His-353 AChE corresponds to the Yt(a) and the rare Asn-353 AChE variant to Yt(b) blood group antigen.

The human BCHE gene (3q26.1-q26.2) includes four coding exons, yielding a 602 amino acids long protein. More than 40 SNPs have been described for BChE. “Atypical” BChE (the dibucain resistant Asp70Gly) shows reduced activity, because Asp70 facilitates the initial binding of positively charged substrates to the active site gorge. The K (Ala539Thr), J (Glu497Val) and fluoride resistant (Thr247Met or Gly390Val) variants also show reduced BChE activities. Also, ~ 20 different “silent” genotypes have been recognized, with 0–2% of normal activity. Inversely, the Cynthiana and Johannesburg variants show higher activity than “usual” BChE.

Post-Transcriptional Modifications

The *ACHE* gene includes sites for alternative splicing of its pre-mRNA product both at the 5' and the 3' ends. Three different carboxy termini exist: the “synaptic” or S variant also called as “tailed”, the “erythrocytic” or E variant and the “readthrough” or R variant. These join the two different N-termini to yield variants with the common or the “extended” N-terminus.

Post-Translational Modifications

Both ChEs undergo several post-translational modifications, including glycosylation and glycosylphosphatidylinositolation (GPI), phosphorylation and carbamylation.

Glycosylation: AChE and BChE carry 3 and 9, respectively, N-glycosylation consensus sequences attaching carbohydrate residues to the core protein via asparagines. Different molecular forms of the enzymes in various tissues, show different number and composition of carbohydrate residues. N-glycosylation at all sites was shown to be important for effective biosynthesis, secretion and clearance of ChEs from the circulation. Altered patterns of AChE glycosylation have been observed in the brain and cerebrospinal fluid of Alzheimer's disease (AD) patients, with potential diagnostic value.

Glycosylphosphatidylinositolation: The GlycoPhosphatidyl Inositol moiety anchor of AChE consists exclusively of diacyl molecular species. Over 85% of the molecular species are composed of palmitoyl, stearoyl and oleoyl. The post-translational process of glypiation takes place in the endoplasmic reticulum, after completion of the polypeptide chain; the newly synthesized protein interacts with a transamidase

complex, which cleaves the chain upstream of the hydrophobic region and adds a preformed GPI anchor to the resulting C-terminal residue.

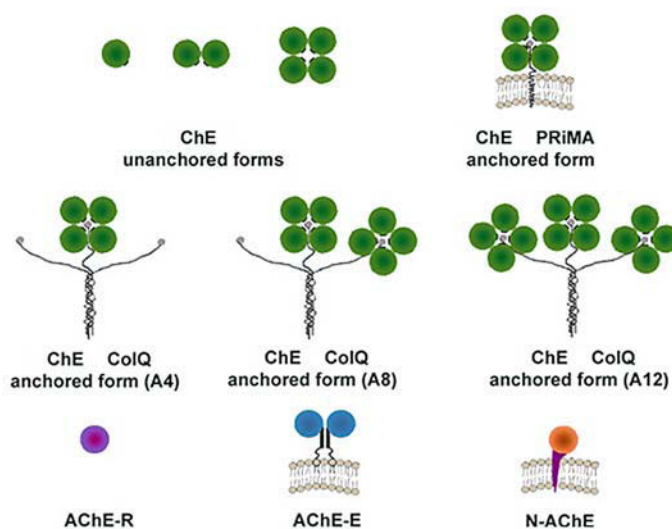
Disulfide bridges formation: ChEs contain 8–10 cysteines; six of these form three internal disulfide bridges. The cysteine that is located four amino acids upstream the carboxyl terminus forms a disulfide bridge with a cysteine of an identical subunit, creating an interchain disulfide bridge, which stabilizes the dimeric structure.

Molecular Forms of ChEs

ChEs present several amphiphilic and soluble homo- and hetero-oligomeric molecular forms in tissues and body fluids, with different tissue distributions (Fig. 1).

BChE and AChE-S (1–4, in green):

1. Amphiphilic monomers and dimers (G1, G2): Abundant in mammalian brain, muscles and intestine for both ChEs.
2. Soluble tetrameric form (G4): Composed of four identical monomers and stabilized by hydrophobic interactions of hydrophobic amino acids at the C terminus of monomers. Abundant for brain AChE and BChE in mammalian body fluids and in the soluble fraction of tissue homogenates.
3. Hydrophobic-tailed tetramers: Abundant form in the mammalian CNS. Anchored to plasma membranes by a hydrophobic, 20 kDalton length polypeptide subunit named PRiMA (Proline-Rich Membrane Anchor).
4. Collagen-like (ColQ) tailed forms or asymmetric multimers: Characterized by triple helical structure of three collagenic subunits Q, each associated with



Cholinesterase. Figure 1 Shown are the seven subgroups of ChEs' Molecular forms: Top, in green: monomeric, dimeric and tetrameric AChE-S forms (G1, G2, G4 and PRiMA). Middle, in green: ColQ tailed AChE-S forms (A4, A8 and A12). Parallel forms exist for BChE. Down: other AChE splicing variants (AChE-R, AChE-E, N-AChE).

one (A4), two (A8) or three (A12) tetramers of ChEs, anchoring to the basal lamina. It is more abundant for AChE than BChE in NMJ.

5. AChE-R (in purple): Naturally rare, stress-induced variant, which lacks a hydrophobic domain and is incapable of binding to ColQ or PRiMA. Therefore, it remains soluble, and its secreted form shows greater mobility than AChE-S. AChE-R can intracellularly interact through its C-terminal tail with the Protein Kinase C Receptor RACK1, a scaffold protein which modifies multiple cellular processes.
6. AChE-E (in blue): GPI-anchored dimers to plasma membranes in mammalian muscles, erythrocytes and lymphocytes.
7. N-AChE (in purple): The N-terminus extension may serve as a transmembrane domain, enabling AChE-R anchorage to the membrane without ColQ/PRiMA. In principle, N-AChE-S, N-AChE-E and N-AChE-R, may exist.

Drugs

Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by progressive cognitive impairment, a variety of neuropsychiatric and behavioural disturbances, and restrictions in activities of daily living. Both AChE and BChE are found in human neurons, glia and in AD's plaques and tangles. AChE activity decreases while BChE activity increases with age (>60 years). The Cholinergic Hypothesis attributes to decreased cholinergic transmission a major role in the expression of cognitive, functional, and possibly behavioural symptoms in AD. Correspondingly, cholinesterase inhibitors (ChEI) became the common treatment for dementia symptoms. Male carriers of both *BCHE-K* and the debilitated apolipoprotein E4 variant, when older than 75 years, show high-risk for late-onset AD in some, but not all, studies.

Another pathway of influence in AD is the facilitation of amyloid- β (A β) aggregation through an interaction with the PAS of AChE but not of BChE. Inversely, the "usual" BChE (and more specifically its C-terminus) was shown recently as to attenuate in vitro the formation of amyloid fibrils [4].

Gulf war syndrome (GWS) is the name given to a variety of psychological and physical symptoms suffered by veterans of the 1991 Persian Gulf War. The symptoms have been remarkably wide-ranging, sometimes somewhat ill-defined. These symptoms were interpreted to reflect exposure to centrally acting anti-AChEs.

Cholinesterase Inhibitors

OPs or carbamate esters are potent inhibitors of both AChE and BChE. These include drugs (e.g. for AD or myasthenia gravis, MG), pesticides, insecticides and

chemical warfare agents. Phosphorylation or carbamylation of the serine hydroxyl group in the substrate-binding domain inhibits the enzyme, causing ACh accumulation in synaptic clefts and over-stimulation of cholinergic receptors. This induces various symptoms, including tension, anxiety, headaches, slurred speech, tremor, convulsions, muscle paralysis, seizure and even death by asphyxiation.

Drugs

ChEIs represent the treatment of choice for AD therapy, including physostigmine and tacrine (of the first generation), donepezil, rivastigmine and galantamine (of the second generation). ChEIs vary widely in their pharmacological profiles and affinities for AChE and BChE. Donepezil and galantamine inhibit AChE by 1000- and 50-fold more than BChE, respectively, whereas rivastigmine inhibits both enzymes with similar affinity.

ChEI treatments have been expanded also to include other dementias and CNS disorders, e.g. delirium, traumatic brain injuries and memory impairments, as well as myasthenia gravis, glaucoma and parasite infections.

Dementia with Lewy bodies (DLB) is considered the second most common cause of dementia after AD. The disorder is characterized by progressive fluctuating cognitive impairment, visual hallucinations and motor features of Parkinsonism. Neocortical cholinergic activity is more severely depleted in DLB than in AD, and DLB also affects the caudate nucleus, the thalamus and the brain stem. Tolerability of ChEI in DLB appears similar to AD, with some gastrointestinal effects and muscle cramps.

Parkinson's disease (PD) patients may suffer cognitive and behavioural impairments including apathy, personality changes and visual hallucinations, with no currently recommended treatment. Their significant cholinergic deficits led to recommendation of ChEI therapeutics.

Vascular dementia (VD) accounts for ~20–30% of dementia cases, with clinical and pathological overlap with AD. Reductions in cholinergic markers suggest cholinergic deficits in VD, and ChEIs increase ACh availability and improve their cerebral blood flow.

Down's syndrome (DS) carriers develop early-onset AD, motivating the use of cholinergic therapy in this disorder.

Traumatic brain injury is the most common cause of death in subjects under the age of 40, and an important risk factor for AD. Loss of hippocampal cells and depletion of ACh and of muscarinic receptors can be attenuated in injured experimental animals, improve blood perfusion in ischemic areas and increase cholinergic transmission in cortex and hippocampus; the same mechanism invoked for treatment of VD.

Delirium, e.g. post-narcotic delirium, somnolence or coma, is a common complication involving dementia, with fluctuating attention and consciousness and considerable morbidity. It is not always reversible and there is no specific treatment. Some of the accompanying central cholinergic syndromes can be reversed by ChEIs.

Myasthenia gravis (MG) is an autoimmune disorder characterized by weakness of face, tongue and/or neck, which result in double vision or drooping eyelids, along with difficulty chewing, swallowing and talking. The most commonly used ChEIs in MG are pyridostigmine, neostigmine and huperzine A. An AChE mRNA-targeted agent, Monarsen, is under clinical trials for ameliorating MG symptoms, with significant advantages over ChEIs.

Chemical Warfare Agents, Pesticides and Insecticides

Discovered in the late 1930s in Germany as improved poisonous insecticides, organophosphorus ChEIs were developed as chemical warfare agents (e.g. sarin, soman, and tabun) and were more recently employed in the 1995 terrorist attack in the Tokyo subway system [5].

Therapy against acute nerve gases toxicity includes pre-treatment with pyridostigmine, a reversible carbamate ChEI, capable of inducing AChE over-expression. Post-exposure treatment is continuous with administration of cholinolytic agents such as atropine, the oxime reactivator, pralidoxime chloride and diazepam. Although multi-drug combination therapy is effective in increasing survival, it must be administered immediately and cannot prevent the occurrence of post-exposure toxic symptoms. A recently developing alternative involves the use of highly purified ChEs as therapeutic agents. Recombinant production systems for such proteins include transgenic goats producing human BChE in their milk, or plants production of human AChE.

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Cholinesterase Inhibitor

Cholinesterase inhibitor (anti-cholinesterase, ChEI) is a chemical that prevents cholinesterases (ChEs) from breaking down. ACh, which consequently increases the level and duration of action of this neurotransmitter. ChEIs such as organophosphates (esters of phosphoric acid) and carbamates (esters of carbamic acid) – serve as insecticides, pesticides, warfare agents and drugs.

► Cholinesterases

Chorionic Gonadotropin

Chorionic gonadotropin (CG) is produced in the placenta. Together with the pituitary hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), it constitutes the glycoprotein family of gonadotropins. The actions of CG are mediated by the LH receptor, both belonging to the superfamily of G-protein Coupled Receptors.

► G-protein-coupled Receptors

► Contraceptives

Choroideremia

Disorder characterized by atrophy of the choroid (the thin membrane covering most of the posterior of the eye between the retina and sclera) and degeneration of the retinal pigment epithelium resulting in night blindness. The disease is caused by mutations in Rab escort protein Rep1 (component A of Rab geranylgeranyl transferase).

► Protein Trafficking and Quality Control

Chromatin

Chromatin is a noncovalent complex consisting of DNA and dedicated packing proteins, the histones. The name chromatin is derived from the Greek word chroma

(Color) since chromatin can be stained by certain dyes and visualized under a microscope. The main purpose of chromatin is the packaging of DNA and regulation of gene activity. Chromatin can be divided into euchromatin and heterochromatin that differ in microscopic appearance and gene activity. Euchromatin is relatively pale on staining and represents low condensed chromatin with active gene expression. Heterochromatin is intensively stained and strongly condensed without or only low gene activity.

Chromatin is composed of nucleosomes, where each comprise 147 base pairs of DNA wrapped around an octamer of two copies of each histone H2A, H2B, H3, and H4. Nucleosomes are folded into higher-order structures that are stabilized by linker histones. Chromatin structure can be altered by enzymes that posttranslationally modify histones (e.g., through phosphorylation, acetylation, methylation, or ubiquitination) or by ATP-driven chromatin-remodeling complexes that alter nucleosome position and/or composition.

► Histone Acetylation

► Transcriptional Regulation

Chromosomal Translocations

The presence of chromosomal translocations is a consistent feature of many leukemia's, lymphomas, and certain solid tumors. At the genetic level, these events can either deregulate an intact gene by disruption or removal and replacement of the adjacent controlling elements, or create a new fusion gene that express the N-terminus of one protein fused to the C-terminus of another protein.

► RNA Interference (RNAi) – siRNA

Chromosome Segregation

Separation of sister chromatids during anaphase of mitosis. Mitotic chromosomes are first aligned on the metaphase plate before the sister chromatids are separated induced by cleavage of proteins, termed cohesins, holding the chromatids together. The cleavage of cohesins requires the ubiquitin-dependent protein degradation mediated by the APC/C.

► Cell Cycle Control

Chromosomes

Double helical DNA is organized in all cells into structures generically referred to as chromosomes. Chromosomes contain most of the genetic information of a cell, although bacterial cells may also have plasmids and eukaryotic cells may also have episomes (extrachromosomal elements). In most bacterial cells, the bacterial chromosome is organized as a single circular double-stranded DNA molecule. Most multicellular organisms have several chromosomes that are linear instead of circular. Sexually reproducing organisms have two copies of each chromosome, one from each parent.

Chronic Bronchitis

Chronic productive cough without a medically discernible cause that is present for more than half the time for 2 years.

► Chronic Obstructive Pulmonary Disease

Chronic Obstructive Pulmonary Disease

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Synonyms

Chronic bronchitis; Pulmonary emphysema

Definition

Chronic obstructive pulmonary disease (COPD) affects over 5% of the adult population, is the fourth leading cause of death worldwide and is the only major cause of mortality that is increasing worldwide. It is an inflammatory disorder of the lungs, caused mainly, but not exclusively, by cigarette smoking. 15–20% of smokers develop COPD.

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) has defined COPD as a disease state characterized by airflow limitation that is not fully

reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases (► www.goldcopd.com). The severity of COPD is classified on the basis of chronic symptoms (cough, sputum production) and spirometric lung function tests.

The definition above has replaced older ones that focused to varying degrees on ► **chronic bronchitis** and/or ► **emphysema**. Chronic bronchitis and emphysema frequently represent different consequences of the same insult leading to changes in large airways, small airways and pulmonary parenchyma (Table 1).

Basic Mechanisms

During COPD, the following symptoms occur, usually in the order mucus hypersecretion, ciliary dysfunction, airflow limitation, pulmonary hyperinflation, gas exchange abnormalities, pulmonary hypertension and cor pulmonale. Acute exacerbations appear to be mainly triggered by bacteria, viruses or environmental pollutants. They lead to a worsening of lung functions, wasting and increased mortality; their psychosocial impacts include depression and anxiety that may be associated with the will to die.

COPD is a chronic inflammatory disease that results from prolonged and repeated inhalation of particles and gases, chronic (or latent) infection or an interaction of these factors. In many cases, the inflammation persists even when the exposure (in most cases smoking) is stopped. Prominent among the infiltrating leukocytes are neutrophils, ► **CD8⁺** lymphocytes (Co-receptor for the T-cell receptor. CD8⁺ is specific for the class I MHC protein. It is expressed on the surface of cytotoxic T-cells and natural killer cells.) and ► **CD68⁺** monocytic cells (A lysosomal antigen. All cells that rich in

lysosomes are CD68⁺, e.g. monocytes, macrophages, mast cells.) (Table 1). The particular contribution of these cell types to the pathology of COPD is under intense discussion. However, because COPD develops over so many years, it remains difficult to distinguish cause and correlation.

One intensively investigated feature of the inflammatory process in COPD is the release of proteases from neutrophils and monocytic cells that destroy elastin and other components of the interstitial matrix (Table 1). The best studied protease is neutrophil elastase. Independent of its elastolytic activity, neutrophil elastase is a potent secretagogue. More recently matrix metalloproteases (MMP) have received increasing attention, in particular MMP12 (macrophages elastase). To which extent and how exactly these proteases become activated is not clear at present.

Both elastase and MMPs have physiological antagonists, named α 1-AT (α 1-anti-trypsin is the primary inhibitor of neutrophil elastase) and TIMP (tissue inhibitor of matrix metalloproteases), respectively. Smoke, presumably through oxidative stress may inactivate these anti-proteases. Consequently, it has been suggested that emphysema results from an imbalance of the protease: anti-protease ratio. And in fact, hereditary α 1-AT deficiency is a rare, but well-known cause of emphysema. In experimental animals, TIMP-3 deficiency leads to a combination of developmental airspace enlargement combined with progressive destructive emphysema in adults. Almost certainly, no single protease/anti-protease alone is responsible for the development of COPD.

Oxidative stress has received much attention as a potentially pathogenic factor. It may promote COPD by many factors such as induction of pro-inflammatory genes in many cells including epithelial and endothelial

Chronic Obstructive Pulmonary Disease. Table 1 Pathological changes in chronic obstructive pulmonary disease (COPD)

	Large airways	Small airways (<2 mm ϕ)	Paren-chyma	Products/consequences
Goblet cell hyperplasia/metaplasia	+ ^a	+	–	Mucus
Neutrophil infiltration	+ ^b	+ ^b	(+) ^b	ROS, elastase, MMP-8, MMP-9
CD8 ⁺ T-cells	+	+	+	TNF, perforins, granzymes, activation of Fas-dependent apoptosis
Macrophages (CD68 ⁺)	–	+	+	TNF, IL-8 cathepsins B, L and S MMP-2, MMP-9, MMP-12
Airway wall fibrosis, thickening	–	+	–	Flow limitation
Smooth muscle	–	+	–	possibly AHR
Emphysema	–	–	+	Flow limitation

ROS, reactive oxygen species; AHR, airway hyperresponsiveness; MMP, matrix metalloprotease.

^aCorrelates with cough and chronic bronchitis.

^bNumber increases with disease severity.

cells, inactivation of anti-proteases or promotion of histone acetylation. ► **Reactive oxygen species** and other factors such as ceramide or blockade of vascular endothelial growth factor (VEGF) receptors may cause emphysema by induction of apoptosis (in the case of reactive oxygen species also necrosis) in endothelial or epithelial cells. Furthermore, inefficient apoptotic cell clearance (efferocytosis) may also contribute to a probably genetically modulated progressive lung destruction that is accompanied with some fibrous scarring.

Today, COPD is regarded as a systemic disease. The different pathomechanisms do not cause only local lung injury and remodelling, but also alter systemic structure and function. Relatively little is known about the low-grade systemic inflammation present in COPD that is considered the main cause for the systemic effects. These systemic effects of COPD include involuntary weight loss, muscle wasting, impaired bone metabolism, reduced functional capacity and health status, and increased cardiovascular morbidity as an important resource-consuming co-morbidity. Thus COPD is multidimensional disease. To account for the multiple changes in COPD the BODE index was introduced, a 10-point scale combining measures of BMI (B), the degree of airflow obstruction (O) and dyspnea (D), and exercise capacity (E).[1,2]

Pharmacological Interventions

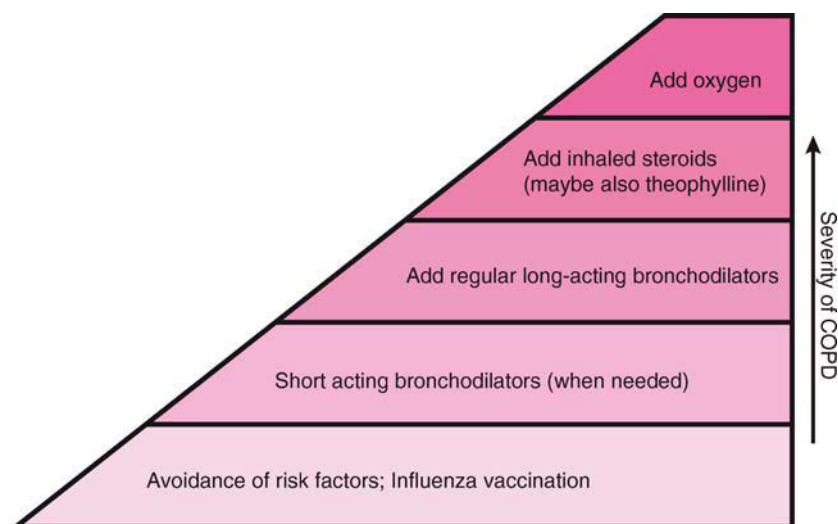
Usually, it takes years of toxin exposure to cause the pathological alterations seen in COPD. In most cases, the disease is already well-progressed when COPD is diagnosed. Reversal of established chronic inflammatory disease is always extremely difficult to achieve and at present healing of COPD is impossible. Smoking cessation is the single most effective and cost-effective

intervention to reduce the risk of developing COPD and to stop its progression. Most of the pharmacological agents that are used in COPD (Fig. 1) have been developed for the treatment of asthma, where their benefit is clearly greater. The comparatively small effect of inhaled β -agonists on airway resistance is even used as a diagnostic criterion to distinguish COPD from asthma. The management of COPD is largely symptom driven, and there is only an imperfect relationship between the degree of airflow limitation and the presence of symptoms. Currently, there is no effective therapy for the irreversible airflow obstruction that results from airway remodelling, fibrosis and emphysema. Available pharmacotherapy can reduce or abolish symptoms, increase exercise capacity, reduce the number and severity of exacerbations, and improve health status. The inhaled route is preferred. Contraindicated for the treatment of stable COPD are anti-tussives and inhaled NO[1,4,5.]

Bronchodilators

The primary aim of the current COPD therapy is to reduce airway resistance by reducing bronchial smooth muscle constriction and mucous plugging. β_2 -adrenoreceptor agonists and anti-cholinergics are the mainstay of therapy for symptomatic management. These bronchodilators improve symptoms, exercise tolerance and may reduce exacerbations, but have little effect on inflammation and on the long-term decline in lung functions. β_2 -agonists and to some extent also anti-cholinergics may increase the risk of adverse cardiovascular events. The combination of bronchodilators with inhaled steroids is probably more effective than either treatment alone.

β_2 -adrenoreceptor agonists: β -agonists increase intracellular cAMP, which in turn leads to bronchodilation and improved lung emptying during breathing.



Chronic Obstructive Pulmonary Disease. Figure 1 Pharmacotherapy of chronic obstructive pulmonary disease (COPD).

Both short- (<6h: salbutamol, terbutaline, orciprenaline, fenoterol) and long-acting (>12h: formoterol, salmeterol) β -agonists are used. Regular treatment with long-acting bronchodilators is more effective and convenient than treatment with short-acting bronchodilators. There is a relatively small and flat dose-response relationship with all β -agonists. Possible side-effects are palpitations and premature ventricular contraction (resulting from stimulation of β_1 -receptors in the heart), tremor and sleep disturbances.

Anti-cholinergics: Tiotropium bromide remains bound to M3-receptors for up to 36 h, and requires only daily intake, whereas other anti-cholinergics (e.g. ipratropium, oxytropium) have to be given up to four-times daily and are often used as maintenance treatment. Possible side-effects are dry mouth, metallic taste after inhalation and very rarely close-angle glaucoma.

Methylxanthines (see also asthma): The best known of these drugs is theophylline. Theophylline is an unspecific phosphodiesterase inhibitor that also possesses some anti-inflammatory effects. Theophylline is generally considered a third-line bronchodilator drug in chronic COPD behind inhaled anti-cholinergics and β_2 -agonists, with slow-release forms of oral theophylline preferred. Methylxanthines have a narrow therapeutic margin. Major side-effects are ventricular and atrial dysrhythmias, and convulsions. Other possible side-effects include headache, nausea, vomiting, diarrhoea and heartburn. More selective phosphodiesterase-4 inhibitors (roflumilast, cilomilast) are under investigation, but have not yet been approved.

Inhaled Corticosteroids

Inhaled steroids (commonly used are beclomethasone, budesonide, triamcinolone, fluticasone, flunisolide) appear to attenuate the inflammatory response, to reduce bronchial hyperreactivity, to decrease exacerbations and to improve health status; they may also reduce the risk of myocardial infarction, but they do not modify the long-term decline in lung function. Whether steroids affect mortality remains unclear. Many patients appear to be resistant to steroids and large, long-term trials have shown only limited effectiveness of inhaled corticosteroid therapy. Certainly, the benefit from steroids is smaller in COPD than in asthma. Topical side-effects of inhaled steroids are oropharyngeal candidiasis and hoarse voice. At the normal doses systemic side-effects of inhaled steroids have not been firmly established. The current recommendation is that the addition of inhaled glucocorticosteroids to bronchodilator treatment is appropriate for patients with severe to very severe COPD.

Mucolytic/Anti-oxidant Therapy

Mucolytic and anti-oxidant drugs include ambroxol, N-acetylcysteine, carbocysteine and iodinated glycerol. These drugs have no proven effect on lung function. At

present, their use is not recommended. N-acetylcysteine may be effective in reducing the number of exacerbations of chronic bronchitis.

Anti-Microbial Therapy

The use of antibiotics is not recommended, except for the treatment of infectious exacerbations of COPD and other bacterial infections. Influenza vaccines decrease illness and death in COPD patients. Pneumococcal vaccination is also recommended.

Psychopharmacological Therapy

Up to 30% of COPD patients suffer from anxiety disorder or depression, and should be treated with conventional pharmacotherapy.

Anti-Trypsin Augmentation Therapy

Approximately 2% of all COPD patients suffer from homozygous α_1 -AT deficiency. Intravenous infusion of replacement protein twice weekly in patients with established α_1 -AT deficiency is approved in the US but not in Europe. The effectiveness of this extremely expensive treatment is not yet known.

Oxygen Therapy

Long-term oxygen therapy (>15 h per day) is introduced in very severe hypoxemic COPD and improves survival, exercise, sleep and cognitive performance. Oxygen therapy is also temporarily used for hospital treatment of hypoxemic COPD exacerbations. In addition to improving oxygenation, oxygen therapy is thought to be effective because it reduces pulmonary hypertension by opposing hypoxic pulmonary vasoconstriction. Hypercapnia is a frequently observed adverse effect after administration of oxygen to COPD patients.

Non-Pharmacological Interventions

Pulmonary rehabilitation as add-on to medication and vaccination has proven to be much more efficient in motivated patients than medication alone. It is aiming at self-management and -training, but also includes nutritional, pharmacological and psychosocial support. Training increases the proportion of type I (minimal fatigable aerobic low-velocity fibres) versus type II fibres (versus the high-velocity anaerobic), stimulates the release of endogenous opioids, provides psychological reinforcement during exercise and improves self-confidence [5].

Lung volume reduction surgery is a rarely performed treatment option for heterogeneous emphysema patients with high hyperinflation. Novel semi-invasive and invasive alternatives are being developed, such as bronchial valves for heterogeneous emphysema treatment, and bronchial stents for homogeneous emphysema to reduce counterproductive collateral ventilation.

Lung transplantation is an ultimate option that has not shown to prolong life, but to improve health status.

- ▶ Bronchial Asthma
- ▶ β -Adrenergic System
- ▶ Muscarinic Receptors
- ▶ Phosphodiesterases
- ▶ Steroids
- ▶ T-cell Receptors

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Chylomicron

Intestinally derived triglyceride-rich lipoprotein.

- ▶ Lipoprotein Metabolism

Chylomicron Remnants

Cholesterol-rich lipoprotein particles that carry dietary lipids absorbed in the intestine and deliver them to the liver for uptake.

- ▶ Low-density Lipoprotein Receptor Gene Family

Chymase

Chymase (mast cell protease type II), a chymotrypsin-like protease, is a serine protease found in mucosal mast cells, which catalyzes the conversion of angiotensin I to angiotensin II and of big endothelin I (ET1) to ET1(1–31).

- ▶ Endothelins

Chymotrypsin-like Proteinases

Chymotrypsin-like proteinases are serine proteinases that recognize peptide residues with aromatic side chains (phenylalanyl or tyrosyl residues) and that effect hydrolysis of the polypeptide chain on the carboxy-terminal side of these residues. Examples of chymotrypsin-like proteinases are chymotrypsin and cathepsin-G.

- ▶ Non-viral Peptidases
- ▶ Cathepsins

Circadian Clock

The phrase circadian clock is used to describe the internal time keeping facilities of an organism, and in animals determines the timing of sleeping and feeding. Most organisms' circadian clocks run to approximately a 24-h cycle, tuned to the light and dark cycle of day and night. Without this light entrainment, for example in conditions of constant darkness, a rhythmic periodicity is still maintained, although the cycle length may deviate from 24-h set-point. In mammals, a brain region called the suprachiasmatic nucleus is responsible for generating the 24-h cycle, with this in turn being regulated by light-generated signals from the retina. The circadian cycle is also modifiable by the relative timing of the availability of food.

- ▶ Circadian Rhythms
- ▶ Sleep

Circadian Rhythms

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Synonyms

Daily rhythms; 24-h rhythms

Definition

Many organisms display prominent variations in their behavioral, metabolic, and biochemical activities. Rhythms that have a period close to 24 h (24-h)

are called ►circadian rhythms (from the Latin “circa” meaning “about” and “dien” meaning “day”). Circadian rhythms are governed by an intrinsic molecular mechanism that is able to generate and sustain periodicities even under constant environmental conditions (i.e., in constant darkness). The circadian clock system can respond to various environmental cues (light, food, etc.) to synchronize the periodicities of the internal rhythms with the environment. The circadian clock system is comprised of a number of proteins that, through complex networks of regulation, determine the expression and function of target genes in both the brain and in peripheral tissues [1].

Basic Mechanisms

Organization of the Mammalian Circadian System

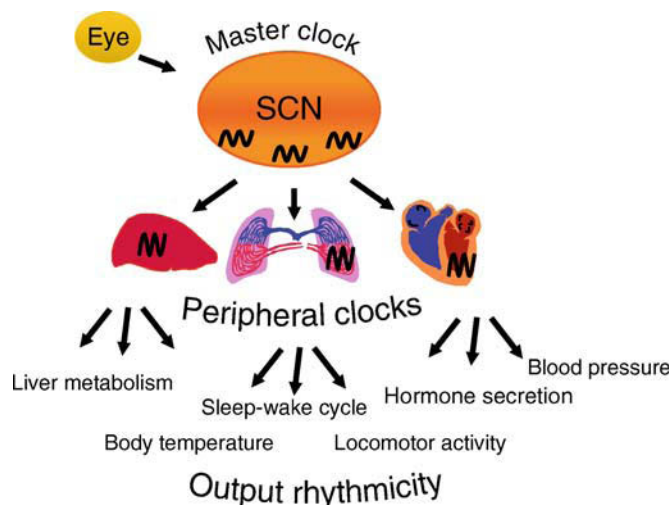
In mammals, the circadian system is organized as a hierarchical network of oscillators or “clocks” (schematically presented in Fig. 1). At the top of this hierarchy is the ►suprachiasmatic nucleus (SCN) of the anterior hypothalamus in the brain. The SCN is comprised of ~20,000 neurons that are able of generating and sustaining a 24-h rhythmicity in electrical and metabolic activities. Being directly connected with a photoreceptive tissue via neural projections, it is reset daily by changes in the environmental light–dark (LD) conditions. Upon synchronizing its phase to the LD cycle, the SCN transmits this information to peripheral clocks that are present in almost every tissue of the body. These peripheral clocks are cell-autonomous and self-sustained as they are capable of persistent rhythmicity independent of SCN input. Thus, at the organismal level, the SCN functions as a phase-coordinator of multiple tissue-specific clocks, rather than as a pacemaker driving peripheral oscillations.

It is believed that upon synchronizing its phase to the environmental LD cycle, the SCN transmits this information to peripheral clocks via a combination of neural and humoral signals. Although the molecular nature of such signals is not completely understood, it is known that there are direct neural projections from the SCN to various hypothalamic and thalamic regions that regulate a variety of other organ functions. In addition, two neuropeptides (TGF β and prokineticin 2) have been identified as factors produced by the SCN that affect other regions of the brain and play roles in controlling the rhythmicity of locomotor activity in mice.

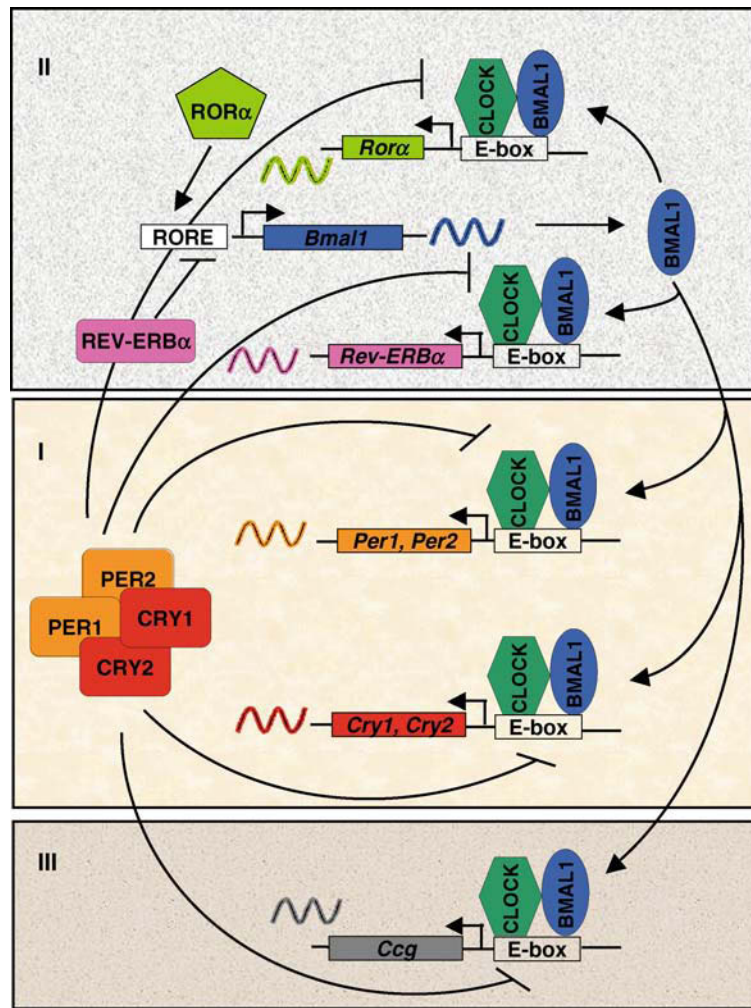
Although under normal conditions the SCN plays a dominant role in synchronizing peripheral clocks to the LD cycle, under special circumstances (for example, an artificial feeding schedule with a phase opposite to the normal LD conditions), the phases of molecular oscillations in the SCN and peripheral tissues (for example, in the liver) can become completely uncoupled from one another. This finding suggests the existence of yet unknown synchronization signaling molecules that function in a tissue-specific manner independent of the SCN. Some examples of known signaling molecules that impact the circadian system include dexamethazone, a glucocorticoid receptor agonist that can reset the phase of peripheral oscillators in the liver, protein kinase C and some chemical signals (cAMP, and Ca²⁺) that can induce circadian gene expression in tissue culture cells.

Molecular Mechanism of Circadian Clock Function

In all types of mammalian cells, the molecular mechanism underlying circadian clock function is based upon interconnected transcription/translation feedback loops [2] (Fig. 2). Two proteins that function as transcriptional



Circadian Rhythms. Figure 1 Mammalian circadian system is organized as a hierarchy of multiple clocks that function to synchronize timing of internal processes between each other and with the environment. See details in the text.



Circadian Rhythms. Figure 2 Simplified schematic representation of the mammalian circadian autoregulatory feedback loops. (I – middle panel). Core circadian autoregulatory loop. Positive elements of the loop (CLOCK and BMAL1) dimerize to activate rhythmic transcription of *Per* and *Cry* genes through E-box enhancer elements. The nuclear-localized CRY and PER proteins interact with CLOCK and BMAL1 to negatively regulate CLOCK: BMAL1-mediated transcription; (II – top panel). Positive transcriptional loop. Transcription of nuclear receptor hormones *Rev-erba* and *Rora* is regulated by the same components that control *Per* and *Cry* transcription, and the resulting proteins compete for the RORE site in the promoter of *Bmal1* gene to periodically repress (REV-ERBα) or activate (RORα) its transcription. (III – lower panel). Molecular basis for clock-controlled processes. CLOCK/BMAL1 complex directly or indirectly regulates transcription of multiple genes via E-box elements in their promoter regions; *Ccg* – clock-controlled genes.

activators, CLOCK and BMAL1, represent the positive elements of the central oscillatory feedback loop. The **CLOCK/BMAL1** (Major circadian transcription complex) heterodimer binds to E-box elements in the promoters of target genes and drives rhythmic transcription of three *Period* (*Per1*, *Per2*, and *Per3*) and two *Cryptochrome* (*Cry1* and *Cry2*) genes. When PER and CRY proteins are translated, they form PER/CRY complexes that are translocated to the nucleus where they inhibit CLOCK/BMAL1-mediated transcription, forming a negative autoregulatory loop. As a result,

transcription of the *Per* and *Cry* genes is reduced, leading to a decrease in the abundance of the corresponding proteins. This alleviates repression of CLOCK/BMAL1 by PER/CRY and leads to initiation of a new transcriptional cycle. CLOCK/BMAL1 also controls transcription of *Rora* and *Rev-erba*, genes encoding two nuclear receptors that compete for the RORE site in the promoter of the *Bmal1* gene. These transcriptional regulators are responsible for either activation (RORα) or repression (REV-ERBα) of *Bmal1* transcription, thus forming an additional feedback loop (Fig. 2). Positive and negative

elements of these regulatory loops function to coordinate transcriptional events at appropriate times to provide 24-h periodicities.

The generation of ~24-h periodicities in expression of **clock genes** is further controlled by various posttranslational modifications of clock proteins. Both positive and negative components of the mammalian transcriptional feedback loops are subject to posttranslational modifications that affect their intracellular distribution and/or stability and, consequently, their function in the molecular clock mechanism. Phosphorylation of PER proteins by casein kinases I epsilon (CKIε) or delta (CKIδ) destabilizes the protein, initiates proteasome-dependent degradation and affects its intracellular distribution. Both CRY1 and CRY2 are phosphorylated in vivo in a time-dependent manner. CLOCK and BMAL1 display circadian oscillations in their phosphorylation status and intracellular distribution. Phosphorylation of CLOCK and BMAL1 is triggered by their coexpression and physical interaction and is closely coupled with the nuclear/cytoplasmic distribution, stability, and transcriptional activity of the CLOCK/BMAL1 complex. In addition, BMAL1 undergoes CLOCK-dependent sumoylation that affects the stability of the protein. Altogether, these modifications introduce temporal delays in transcriptional inhibition ensuring circadian rhythms maintain periodicities close to 24-h.

Clock-Controlled Processes

In addition to core components of the circadian oscillatory machinery, such as the *Per*, *Cry*, and *Rev-erba* genes, the CLOCK/BMAL1 complex also drives rhythmic expression of numerous output genes harboring E-boxes in their promoter regions. These genes, which are called clock-controlled genes, encode proteins that are involved in various tissue-specific metabolic and regulatory pathways. Thus, periodic activation/repression of numerous **clock-controlled genes** provides the mechanistic basis for circadian control of output physiology and metabolism. As revealed by several gene expression profiling studies, a significant portion of the genome (~10% of all transcripts) demonstrates 24-h periodicity in its expression pattern. These cycling transcripts are regulated either directly via CLOCK/BMAL1-dependent activation or indirectly by other transcription factors whose expression is under clock control. Importantly, clock-controlled genes are expressed in a tissue-specific manner with very little overlap between different tissues. In many cases, tissue-specific clock-controlled genes have been shown to be involved in rate-limiting steps of processes critical for the function of an organ. For example, some of the genes under circadian regulation in the SCN are involved in protein/neuropeptide synthesis, processing and degradation or in regulation of redox state and

energy utilization. In the liver, the major metabolic organ in mammals, coordinated circadian expression of genes encoding components of sugar, lipid, cholesterol, and xenobiotic metabolic pathways has been reported. These examples demonstrate how oscillations in core clock genes and multiple tissue-specific target genes together account for the circadian rhythmicity of physiological processes and their coordination with the environment [3].

Circadian-Related Disorders

Given the important role that the circadian system plays in timing various physiological processes in concert with daily environmental changes, it is likely that either dysfunction of the internal clock or disruption of its synchronization with the environment could affect an organism's health. This has been most clearly demonstrated by the involvement of clock genes in susceptibility to sleep disorder syndromes. Mutations in the human circadian genes *Per2* and have been linked to Familial Advanced Sleep Phase Syndrome, a disorder in which the major sleep episode is advanced in relation to the desired time of day. Structural polymorphism in another human clock gene, *Per3*, has been related to the pathogenesis of another common sleep disorder, Delayed Sleep Phase Syndrome.

Desynchronization of various tissue-specific rhythms at the level of an organism may also cause serious pathological conditions. Thus, people who work in rotating shifts or at night have been shown to experience a higher incidence of heart disease, back pain, respiratory problems, ulcers, and sleep disorders. These people also have higher rates of error and accident and often experience a significant loss of alertness and ability to make decisions. Another common example of rhythm disruption is induced by travel between time zones, which often results in jet lag, a less serious, but often debilitating and disorienting state. The importance of circadian rhythms in human biology is further supported by numerous observations demonstrating that some widespread pathological conditions display a strong circadian component (for example, the increased occurrence of heart attacks in the morning or asthmatic attacks during the night hours). Finally, both in animal model systems and in human clinical settings, it has been demonstrated that the sensitivity of normal cells and tissues to chemotherapeutic drugs and radiation depends upon the functional status of the circadian clock system.

However, despite its enormous importance to human physiology, no pharmacological compounds targeting the components of the circadian clock system have been identified to date. There are, nevertheless, two therapeutic approaches that are currently used for treatment of circadian-related disorders – full-spectrum and bright light therapy and melatonin therapy. Melatonin is a hormone that is produced by the pineal gland in

circadian fashion. Its secretion peaks in the middle of the night, gradually falls during early morning hours. Applications of both light and melatonin, appropriately timed, have been shown to induce phase-shifts in human circadian rhythms, presumably acting through the SCN. In addition, both treatments have acute physiological and behavioral effects. Depending upon the dose, melatonin (given during the day) can reduce core body temperature and induce sleepiness. Conversely, light exposure during the night increases body temperature and enhances alertness and performance. However, there is currently no melatonin formulation approved for clinical use, nor have consensus protocols for either therapy been developed. Similar to the SCN, which is directly regulated by light exposure, peripheral oscillators in nonphotoreceptive tissues can be reset by a variety of chemical signals. However, all of the presently known circadian modulators (TGF β , glucocorticoids, calcium ionophores, inhibitors and activators of protein kinases, glucose) have an extremely broad spectrum of action, affecting many cellular and physiological processes. This severely restricts their usefulness as specific regulators of circadian behaviors.

Circadian Clocks and Modulation of Therapeutic Responses

In addition to regulating an organism's physiology under normal conditions, circadian proteins play an important role in modulating responses to various stress conditions including those associated with DNA damage induced by anticancer agents such as chemotherapeutic drugs and radiation. Both experimental and clinical studies have conclusively demonstrated that in vivo responses to anticancer therapies show significant daily variations and correlate with the functional status of the CLOCK/BMAL1 transcriptional complex [4].

Although the exact mechanism underlying circadian control of genotoxic stress (Cellular response to various DNA-damaging agents that activate multiple pathway leading to cell death or cell survival.) is not completely understood, there are several lines of observation suggesting that it involves complex, multilevel regulation. Indeed, global gene expression analysis of circadian transcriptional output demonstrated that many genes encoding drug-metabolizing enzymes display 24-h periodicities in their expression patterns in the liver and other tissues. Moreover, circadian rhythms have been observed in the proliferation of hematopoietic cells, vascular endothelial cells and intestinal epithelial cells. Since cell sensitivity to genotoxic stress depends strongly upon cell cycle stage, this might also impact time-of-day variations in drug and radiation responses. The CLOCK/BMAL1 complex may also be directly involved in regulating the expression of apoptosis-related genes as indicated by low-amplitude circadian variations in the expression of some pro- and

antiapoptotic genes in mouse bone marrow and tumors. It is also possible that extracellular signaling molecules such as cytokines, growth factors, and hormones might affect cell survival following genotoxic stress. Some of these agents, such as fibroblast growth factor, epidermal growth factor, and transforming growth factor display daily variations in their plasma concentrations. Thus, it is likely that circadian modulation of responses to genotoxic stress in vivo reflects the complex superposition of multiple levels of regulation involving a number of factors. An improved understanding of the molecular mechanisms underlying clock-controlled responses to a given treatment may ultimately lead to development of strategies to improve therapeutic efficacy.

► Orexins

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Circumventricular Organs

The circumventricular organs are brain structures bordering the third and fourth ventricles and are unique since they lack a blood–brain barrier. Therefore, they are recognized as important sites for communication between the brain and peripheral organs via blood-borne products. They include the median eminence, subfornical organ, area postrema, subcommissural organ, and organum vasculosum.

Cl[−] Channels and Cl[−]/H⁺ Exchangers

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Synonyms

Anion channels

Definition

Chloride channels are membrane proteins that allow for the passive flow of anions across biological membranes. As chloride is the most abundant anion under physiological conditions, these channels are often called chloride channels instead of anion channels, even though other anions (such as iodide or nitrate) may permeate better. As some CLC proteins function as Cl⁻-channels, whereas other perform Cl⁻/H⁺-exchangers are also mentioned here.

Basic Characteristics

Chloride channels are transmembrane proteins with several transmembrane domains, which form a pore that allows for the passive flow of anions along their electrochemical gradient. Like other channels, chloride channels can be opened or closed by a process called gating. Gating can be influenced by several factors, e.g. by the transmembrane voltage in voltage-gated chloride channels, by intracellular Ca in Ca-activated chloride channels, by extracellular ligands such as glycine or GABA as in ligand-gated chloride channels, by cAMP-dependent phosphorylation, or by cell swelling. Chloride channels may be present in the plasma membrane or in the membranes of intracellular organelles. Several intracellular CLC proteins that were previously thought to be Cl⁻ channels recently turned out to rather be electrogenic Cl⁻/H⁺ exchangers. They will be included in this entry.

Classification of Chloride Channels

Chloride channels can be classified by their biophysical characteristics (e.g. single-channel conductance), regulation (e.g. voltage-dependent, ligand-gated, swelling-activated, Ca-activated), or by their sequence (gene families). The latter classification is the most logical one. However, many classes of chloride channels characterized in native tissues have not yet been cloned, raising the possibility that entire gene families of chloride channels remain to be discovered. Therefore, a molecular classification does not yet cover all chloride channels.

Gene Families of Chloride Channels

There are three well established molecular classes of chloride channels: ►CLC chloride channels (several CLC proteins, however, are Cl⁻/H⁺ exchangers), ligand-gated chloride channels (GABA- and glycine-receptors), as well as ►CFTR, the cystic fibrosis transmembrane conductance regulator which belongs to the ABC-transporter family. There is also rather strong evidence that bestrophins are Ca⁺⁺-activated Cl⁻ channels. Other proposed gene families include the CLIC proteins and the CaCC proteins, whose function as chloride channels, however, has not yet been proven [1].

CFTR is the only member of the very large ABC-transporter gene family that is known to function as a

chloride channel. Most other members of this gene family (like *mdr*) probably function as ATP-dependent pumps and not as channels. CFTR is regulated by intracellular ATP and cAMP. cAMP acts through phosphorylation by protein kinase A, resulting in a cAMP-activated chloride channel. CFTR is expressed in many epithelia, e.g. in apical membranes in the lung, pancreas and intestine. Mutations in CFTR underlie cystic fibrosis, a potentially lethal disease with transport defects in the lung, pancreas and colon. In addition to working as a chloride channel, several other important functions (such as the regulation of other ion channels or being a receptor for bacteria) have been attributed to CFTR. CFTR has 12 transmembrane domains and may function as a monomer.

GABA_A- and glycine-receptors are ligand-gated chloride channels that belong to a gene superfamily that also includes cation channels (e.g. nicotinic acetylcholine receptors). These ion channels are involved in synaptic transmission and are mostly inhibitory in the adult due to the direction of the chloride concentration gradient. These ligand-gated channels are important pharmacological targets, but cannot be discussed in detail here. These channels function as pentamers of identical or homologous subunits, with four transmembrane spans each.

CLC chloride channels form a large gene family with members in bacteria, archae and eukaryotes. Many CLCs gate in a voltage-dependent manner. In mammals, there are nine different encoding CLC proteins. CLC channels are present in the plasma membrane and in intracellular organelles. They function as dimers, with each monomer having its own pore ("double-barreled" channels). The structure of a bacterial CLC protein has recently been determined by X-ray crystal structure analysis [2]. Some CLC channels have accessory β-subunits [3]. The CLC protein from *E. coli*, as well as the mammalian endosomal CIC-4 and CIC-5, were recently shown to be Cl⁻/H⁺ exchangers and not channels [4].

The CLC Family of Chloride Channels and Transporters in Mammals

There are nine different CLC isoforms in mammals. Based on homology, they can be classed into three branches. The first branch includes channels that reside predominantly in the plasma membrane. This includes CIC-1, a skeletal muscle chloride channel, CIC-2, a very broadly expressed channel, and CIC-Ka and CIC-Kb, which are expressed predominantly in the kidney but also in the ear.

The physiological roles of these channels are apparent from human diseases or mouse models in which these genes are disrupted.

Mutations in CIC-1 lead to ►myotonia, a muscle stiffness that is associated with a hyperexcitability of the muscle plasma membrane. Thus, the high resting

chloride conductance in muscle is necessary for its electrical stability. CIC-1 shows a distinct voltage-dependence and is activated by depolarization. It is blocked in a voltage-dependent manner by iodide.

The disruption of CIC-2 in mice leads to male infertility, blindness, and leukodystrophy, and was attributed to defective extracellular ion homeostasis in narrow clefts. CIC-2 yields currents that slowly activate upon hyperpolarization. It is also activated by cell swelling and by extracellular acidification. Structural determinants that are essential for these types of activation were identified by mutagenesis. There is a report that CIC-2 might be mutated in human epilepsy, but this has not been confirmed in further studies.

Mutations in CIC-Kb lead to ►**Bartter's syndrome**, a disease associated with severe renal salt loss. This demonstrates that CIC-Kb is essential for the basolateral efflux of chloride from cells of the renal thick ascending limb of Henle. The disruption of CIC-K1 (the species *orthologue* of CIC-Ka) in mice leads to a syndrome resembling nephrogenic diabetes insipidus, and it was shown that it is essential for the establishment of high osmolarity in kidney medulla. CIC-Kb and CIC-Ka need barttin, a protein with two transmembrane domains, as a β -subunit for functional expression. Mutations in barttin lead to Bartter syndrome with deafness. It has been shown that barttin associates with CIC-Ka and CIC-Kb in the basolateral membrane of the stria vascularis of the inner ear. In this tissue, CIC-K/barttin heteromeric channels are necessary for the basolateral recycling of chloride that is taken up by a basolateral NaK2Cl cotransporter. Barttin mutations lead to deafness because K-secretion by the stria into the scala media of the cochlea is impaired. In the kidney, CIC-Ka/barttin channels are present in the thin limb of Henle's loop, while CIC-Kb/barttin is present in the thick ascending limb of Henle's loop and some more distal segments (e.g. acid-secreting intercalated cells). Currents of both CIC-Ka/barttin and CIC-Kb/barttin show a rather linear voltage-dependence, are augmented by raising extracellular Ca, and inhibited by extracellular acidification. Given the important role of CIC-K/barttin in renal salt and fluid reabsorption, they are attractive candidate targets for the development of diuretics. In contrast to diuretics that target channels or transporters of apical membranes of the nephron (such as amiloride or furosemide), drugs inhibiting CIC-K/barttin may also be useful in conditions of renal failure.

All members of this CLC branch have a $\text{Cl}^- > \text{I}^-$ permeability and conductance sequence.

CIC-3, -4 and -5 form the second branch of the CLC gene family. These proteins are 80% identical, and with the exception of CIC-5, which is most highly expressed in kidney and intestine, show a broad expression pattern. CIC-3 to CIC-5 reside in intracellular membranes of the endocytotic pathway [4]. Disruption of CIC-5 leads to a defect in endocytosis in mouse

models as well as in human Dent's disease, a disorder associated with proteinuria and kidney stones. CIC-5 currents are necessary to balance the current of the electrogenic proton pump of endosomes. Therefore, the disruption of CIC-5 leads to a defect in endosomal acidification, which impairs endocytosis. The defect in proximal tubular endocytosis leads to secondary changes in calciotropic hormones, leading to tertiary changes such as hyperphosphaturia, hypercalciuria and kidney stones. Recent data show that CIC-4 and CIC-5 are electrogenic Cl^-/H^+ exchangers rather than Cl^- channels, which is still compatible with a role in acidifying endosomes [4].

Similar to CIC-5, CIC-3 is present in endosomes. It is also found in synaptic vesicles. In both instances, and similar to CIC-5, it is necessary for the efficient intravesicular acidification. The acidification of synaptic vesicles is particularly important as their uptake of neurotransmitters depends on the electrochemical proton gradient. Surprisingly, the disruption of CIC-3 in mice resulted in a drastic degeneration of the hippocampus and the retina. Much less is known about CIC-4, which, however, also appears to be present in endosomal compartments.

All three members of this branch give currents with a $\text{NO}_3^- > \text{Cl}^- > \text{I}^-$ conductance sequence. Currents are very strongly outwardly rectified (opening at voltages more positive than +20 mV), which is enigmatic as this voltage range seems not to be attained in vivo. Their currents can be inhibited by extracellular (intravesicular) acidification. The fact that currents can be observed shows that CIC-3, -4 and -5 are not exclusively present in endosomes, but can also come to the surface upon heterologous expression. It is currently unclear whether this occurs under physiological conditions as well.

CIC-6 and CIC-7 define the third branch of the CLC family. These proteins are only about 45% identical to each other. Whereas CLC-7 is very broadly expressed, the CLC-6 protein seems to be restricted to the nervous system. It proved impossible to obtain plasma membrane chloride currents with either CIC-6 or CIC-7. This is due to the fact that both channels reside in intracellular organelles under most circumstances. Based on structural features, it appears likely that they also mediate Cl^-/H^+ exchange.

CIC-6 is a late endosomal chloride transporter. Its disruption in mice led to lysosomal storage disease. CIC-7 is expressed in late endosomes and lysosomes. It needs Ostml as β -subunit [3]. The disruption of either CIC-7 or Ostml in mice and man leads to severe ►**osteopetrosis**, retinal degeneration, and a severe lysosomal storage disease. CIC-7/Ostml is highly expressed in osteoclasts. In these cells, it is inserted together with the proton pump into the specialized plasma membrane ("ruffled border") that faces the reabsorption lacuna. ►**Osteoclasts** are still present in CIC-7 knockout

mice and can still attach to bone. However, they cannot acidify their reabsorption lacuna, resulting in a severe defect of bone resorption. Thus, similar to the roles of other intracellular CLCs, CIC-7 is essential for acidification of certain compartments by electrically balancing the current of the proton pump. CIC-7 may be an interesting target for the treatment of ►osteoporosis as its partial inhibition might increase bone mass. This notion is indirectly supported by the observation that patients that are heterozygous for dominant negative CIC-7 mutations (a situation expected to lead only to a partial inhibition of CIC-7) present with a milder form of osteopetrosis in which retinal degeneration is absent.

Bestrophins

The newest molecular addition to Cl^- channels are bestrophins [5]. There are four different bestrophin isoforms in humans. Mutations in Best1 cause Best macular dystrophy, hence their name. All four isoforms induce chloride currents when expressed heterologously. Although they show a dependence on intracellular calcium, their biophysical properties differ from Ca-activated chloride currents typically observed in native cells. Mutagenesis experiments changed the activation of currents and induced slight changes in ion selectivity, lending support to the hypothesis that bestrophins themselves are Cl^- channels.

Drugs

Unfortunately, the pharmacology of chloride channels is poorly developed. Specific and highly useful inhibitors or modulators (e.g. strychnine, picrotoxin, diazepam) are only available for ligand-gated chloride channels (but these are covered in a different chapter). There are several “chloride channel inhibitors” such as the stilbene-disulfonates DIDS and SITS, 9-antracene-carboxylic acid (9-AC), arylaminobenzoates such as DPC and NPPB, niflumic acids and derivatives, sulfonylureas, and zinc and cadmium. All of these inhibitors, however, are not very specific. Several of these inhibitors (e.g. DIDS) inhibit many chloride channels only partially even at millimolar concentrations and have effects on other types of transport proteins.

Tamoxifen and DIDS have been used to inhibit endogenous swelling-activated chloride channels whose molecular identity is still unclear. Glibenclamide has been used to inhibit CFTR, which is quite resistance to DIDS. Endogenous (probably not yet cloned) Ca-activated chloride channels are often sensitive to fenamates such as flufenamic acid and niflumic acid. CLC channels are quite insensitive to DIDS, but can often be inhibited by zinc or cadmium in a submillimolar range. 9-AC is a quite specific inhibitor for the muscle channel CIC-1, and its inhibitor binding site has been mapped recently by mutagenesis. CIC-1 can also be inhibited by clofibrate acid derivatives.

Lubiprostone, a drug used for treating obstipation, has been claimed to be an activator of CIC-2. This is based on a single paper showing activation by lubiprostone of currents thought to represent CIC-2. These currents, however, differ starkly from typical CIC-2 currents. Furthermore, CIC-2 is located in basolateral membranes of the intestine. This localization is incompatible with the hypothesis that its activation increases intestinal chloride and fluid secretion. Thus, the claim that lubiprostone is a Cl^- channel activator must be subject to considerable doubt.

►Table Appendix: Membrane Transport Proteins

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Clathrin

Clathrin is a protein complex composed of three heavy and three light chains, which assemble in a so-called triskelion. Clathrin is a major constituent of endocytic vesicles.

►Intracellular Transport

Clathrin-coated Pits

Specialized regions of internalization from the plasma membrane, coated with a polyhedral lattice of the protein clathrin. It is in these regions that the first step of the process of endocytosis takes place, with the formation of clathrin-coated endocytic vesicles.

►Intracellular Transport

Clathrin-coated Vesicle

Clathrin-coated vesicles mediate transport within the late secretory and the endocytic pathways. Their major coat constituents are clathrin and various adaptor complexes.

► Intracellular Transport

CLC

CLC is a gene family of CL-channels. Originally identified by the expression cloning of CLC-0 from the electric organ of the marine ray Torpedo, CLC genes are now known to be present in all kingdoms of life, with nine genes in humans alone. CLC proteins have 17 helices in the membrane plane, several of which, however, do not cross the width of the membrane and therefore do not qualify as transmembrane domains. In CLCs of higher organisms (and also in some, but not all bacteria) CLCs have a large cytoplasmic tail with two conserved CBS domains of largely unknown function. CLC channels function as dimers with two largely independent pores ("double-barreled" channel). Each pore is entirely contained within each monomer, and not at the interface between these. All CLC channels that could be functionally expressed have a $Cl^- > I^-$ conductance sequence. Gating is often voltage-dependent and depends on anions, resulting in a model in which anions serve as the gating charge.

► Cl^- Channels and Cl^-/H^+ Exchangers

Clearance

Volume that is cleared from a drug within a defined time interval.

► Pharmacokinetics

Clinical Trials

Clinical trials are usually divided into three phases. In phase I clinical trials, the new drug is tested in a small (20–80) group of healthy volunteers to evaluate its

safety, determine a safe dosage range, and identify side effects. These studies also determine how the compound is absorbed, distributed, metabolized and excreted, as well as the duration of its action. Phase II trials are performed on larger group of people (20–300) to assess clinical efficacy of the therapy, as well as to continue evaluation of the safety and effectiveness of the drug. Phase III studies are randomized controlled trials on large patient groups (300–3,000 or more depending upon the condition) and are aimed at being the definitive assessment of the efficacy of the new therapy.

Clock

Clock gene and transcription factor with histone acetyltransferase (HAT) activity that (in complex with BMAL1) constitutes a positive limb of molecular circadian oscillators.

► Circadian Rhythms ► Orexins

Clonal Selection

T- and B-lymphocytes are the only cells in the body which carry antigen receptors on their plasma membrane. Each individual lymphocyte possesses one type of antigen receptor with specificity for one antigen, which has been created during ontogeny of the cell by irreversible random genetic rearrangement of certain sequences of the DNA. Thus a very large diversity of antigen specificities (possibly $> 10^8$) is generated, which allows the specific immune system to cope with all potentially harmful pathogens or substances in the environment. Pathogens invading an organism "select" (are recognized by) one of the millions of lymphocytes which is then activated in a tightly controlled process and starts to proliferate. It clonally expands in order to create enough T- or B-lymphocyte effector cells capable of coping with the pathogen.

► Immune Defense

Clostridial Neurotoxins

Clostridial neurotoxins are bacterial protein toxins that consist of a heavy and a light chain connected by a disulfide bond and non-covalent interactions. They

include tetanus toxin (TeNT) and the seven serotypes of botulinum toxins termed BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F and BoNT/G. The heavy chain binds to the surface of peripheral neurons and mediates cell entry. After endocytotic uptake, the light chain is released into the cytoplasm. The difference between the clinical effects of tetanus and botulinal neurotoxins is due to the fact that tetanus toxin, while being sequestered by peripheral motoneurons in parallel with the BoNTs, is only inefficiently released into the cytoplasm but rather transported retrogradely into the spinal cord. There it is released by the dendrites of the motoneurons and inhibits predominantly presynaptic glycinergic interneurons, causing overexcitation and muscle cramps.

The light chains of the clostridial neurotoxins are metalloproteases with exclusive specificity for neuronal SNAREs. TeNT, BoNTs B,D,F, and G cleave synaptobrevin, BoNTs A and E SNAP-25, and BoNT/C1 syntaxin, and to a lesser extent also SNAP-25. Cleavage of any of the SNAREs causes complete and irreversible block of synaptic transmission.

- Bacterial Toxins
- Exocytosis
- Synaptic Transmission

Clostridium Botulinum Toxin

- Bacterial Toxins

Clotting

- Coagulation/Thrombosis

CNTF

Ciliary Neurotrophic Factor.

- Neurotrophic Factors

CO

Carbon Monoxide.

Coactivator and Corepressors

Multiprotein complexes that do not directly bind DNA, but are recruited by sequence-specific transcription factors and mediate their capacity to activate genes (coactivators) and to repress genes (corepressors).

- Transcriptional Regulation
- Nuclear Receptors
- Selective Sex Steroid Receptor Modulators
- Contraceptives

Coagulation/Thrombosis

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Synonyms

Blood clotting; Hemostasis; Vessel occlusion

Definition

Coagulation summarizes the mechanisms involved in stopping bleeding due to an injured or defective vessel wall. Coagulation is characterized by procoagulatory and anticoagulatory factors that are in balance under normal conditions. Vessel injuries are occluded by the coagulation system and spontaneous vessel occlusions dissolved by the fibrinolytic cascade.

Thrombosis is an imbalance toward the clotting capacity of the blood that leads to vessel occlusion by a clot. The clot prohibits further blood flow and can so cause pathological sequelae dependent on its localization.

Mechanism of Action

The different pro- and anticoagulatory systems are complex regulated cascades involving blood cells (platelets, monocytes, endothelial cells), enzymes, cofactors, phospholipids, and calcium, which interact with each other

and are further influenced by physical conditions such as blood flow velocity, turbulences, or viscosity.

Coagulation Factors

Coagulation factors are glycoproteins named by roman numbers (the numbers being ascribed at the time of the components' definition, not sequence of activation) (Table 1). Besides von Willebrand factor (vWF), the coagulation factors are synthesized in the liver. They have very different half-lives and different concentrations in the plasma. Several coagulation factors are stored in platelets and endothelial cells and can be released during activation of these cells, which can result in a much higher local concentration of the respective factor (e.g., vWF).

Besides FXIII, all clotting enzymes are serine proteases (FII, FVII, FIX, FX, FXI, FXII). They usually circulate in their inactive form (proenzyme) and become only activated during clotting. In this respect, FVII is an exception as about 1% of FVII circulates in its active form, FVIIa, in plasma without activation of the clotting cascade. Its enzymatic activity is strongly enhanced by binding to its cofactor tissue factor, which under normal conditions is not exposed on cells having direct contact with blood. FV and FVIII are not enzymes but they are required as cofactors to form complexes with activated factors X and IX, respectively.

FVIII circulates in blood complexed to vWF. In the absence of vWF, or in case of impaired binding to vWF, FVIII is degraded.

Activation of the Coagulation Cascade

During activation of the coagulation cascade, coagulation factors form multimolecular (often trimolecular)

complexes. The appropriate sterical orientation of the complex partners is usually provided by a surface of negatively charged phospholipids. The complexes consist of the enzyme, its cofactor, and the respective substrate.

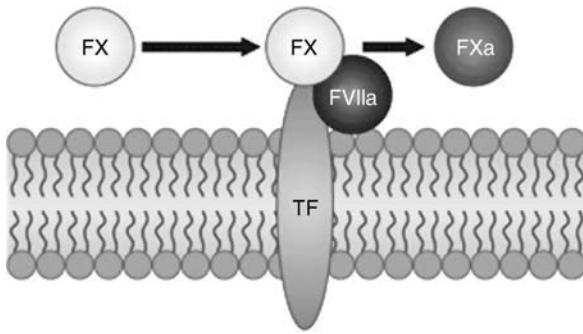
The FVIIa/TF-complex is the main activator of the clotting cascade. Under normal conditions TF is not exposed on the surface of cells being in contact with blood but is expressed in high concentrations by all other cells. When flowing blood comes into contact with TF-bearing cells, TF binds the already circulating FVIIa (Fig. 1). This complex activates the serine proteases FX and FIX, and autocatalyzes activation of FVII (Fig. 2). On the surface of negatively charged phospholipids FXa together with its cofactor Va and the zymogen prothrombin (FII) form the prothrombinase complex, which results in the generation of FIIa (thrombin) (Fig. 3). Within this complex, the activity of FXa to generate FIIa is 300,000× enhanced as compared to the activity of uncomplexed FXa. As activated platelets provide a phospholipid surface rich in negatively charged phospholipids, and as platelets release the reaction determining FV during activation, platelets and clotting factors both contribute to the amplification of the clotting cascade. Activated FIX enhances the initial activation of the clotting cascade by formation of a FX-activating complex together with its cofactor VIII. The importance of this amplifier loop is demonstrated by the bleeding tendency of patients showing inherited FVIII- (hemophilia A) or FIX-deficiencies (hemophilia B). Thrombin by itself contributes to its self-amplifying loop by activating FXI, FVIII, and FV.

Formerly, the clotting cascade had been divided into an extrinsic and intrinsic pathway. Although useful for

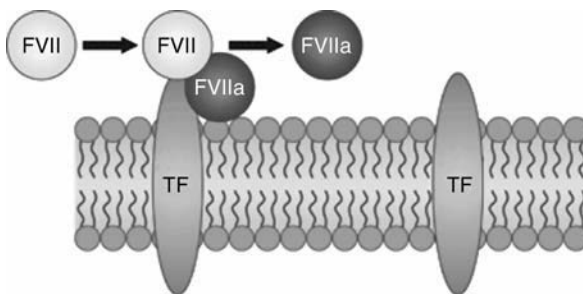
Coagulation/Thrombosis. Table 1 Coagulation/Thrombosis

Name	Abbreviation	Molecular weight	Plasma concentration [mg/l]	Plasma concentration [mmol/l]
Fibrinogen	–	340,000	3000	8800
Prothrombin	–	72,000	100	1400
Factor X	FX	56,000	10	180
Factor IX	FIX	56,000	5	90
Factor VII	FVII	50,000	0.5	10
Factor VIII	FVIII	330,000	0.1	0.3
Factor V	FV	330,000	10	30
Factor XI	FXI	160,000	5	30
Factor XII	FXII	80,000	30	400
Von-Willebrand-factor	vWF	225,000 ^a	10	40
Tissue factor	TF	37,000	0.0	–
High molecular kininogen	HK	110,000	70	600
Prekallikrein	PreKK	88,000	40	500

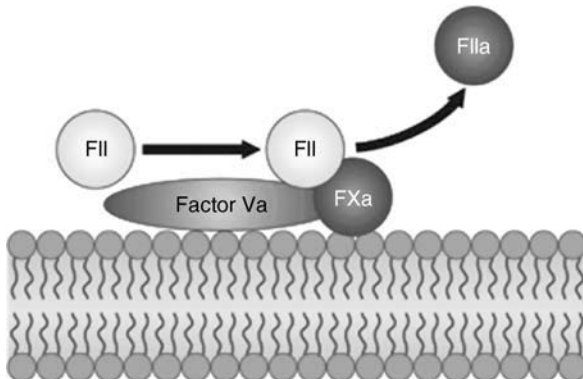
^aMolecular weight of the smallest subunit.



Coagulation/Thrombosis. Figure 1 Coagulation/Thrombosis.



Coagulation/Thrombosis. Figure 2 Coagulation/Thrombosis.



Coagulation/Thrombosis. Figure 3 Coagulation/Thrombosis.

didactic purposes, both pathways are not separated but linked by tissue factor/FVIIa activation (Fig. 4).

While the extrinsic pathway starts with FVIIa binding to TF, the intrinsic pathway is characterized by activation through binding of contact factors (Factor XII, Prekallikrein, high molecular weight kininogen) to negatively charged surfaces. This leads to a conformational change and activation to FXIIa and kallikrein.

Factor XIIa is a serine protease that activates FXI to FXIa (Fig. 5). This system is not of physiologic relevance since patients with hereditary deficiencies of factor XII, prekallikrein, and high-molecular weight kininogen do not present with bleeding symptoms.

Vitamin K-dependent Coagulation Factors

Factors VII, IX, X, and II belong to the group of vitamin K-dependent coagulation enzymes. This group of enzymes binds to phospholipid surfaces via Gla-domains, which are generated in the molecule by γ -carboxylation of glutamic acid residues (Fig. 6). This molecular mechanism explains the therapeutic principle of vitamin K-antagonists, such as warfarine or other coumarines. However, the synthesis of the anticoagulant proteins C and S is also vitamin K-dependent (for clinical consequences see paragraph protein-C-pathway).

Generation of Fibrin

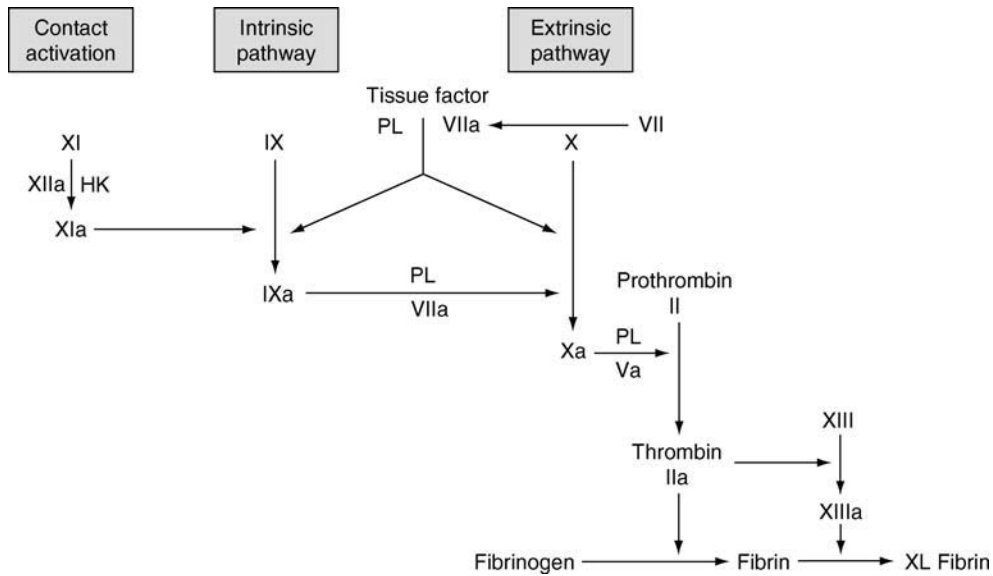
Fibrin is a high molecular polymer. The liver synthesizes its precursor, the protein fibrinogen. It consists of three peptide chains (α -, β -, and γ -chain). Catalyzed by thrombin, the N-terminal fibrinopeptides A and B are cleaved from the α - and β -chain. The remaining peptide chains undergo a conformational change that allows end-to-end polymerization of the fibrin monomers. Stabilization of the fibrin monomers requires cross-linking by the formation of covalent links between the γ - and the α -chains. These links are catalyzed by the transglutaminase factor XIIIa (FXIIIa). FXIIIa is generated from FXIII by thrombin. Fibrin is also linked by FXIIIa to subendothelial proteins like collagen and fibronectin and to adhesive proteins such as vWF and vitronectin. Both, the covalent crosslink within the polymer and the interaction of the fibrinpolymer with the vessel wall provide a stable clot. Noteworthy, patients with a FXIII deficiency present with an intact primary haemostasis but bleeding complications after a time delay of several hours due to instable clot formation.

Regulation of the Clotting Process

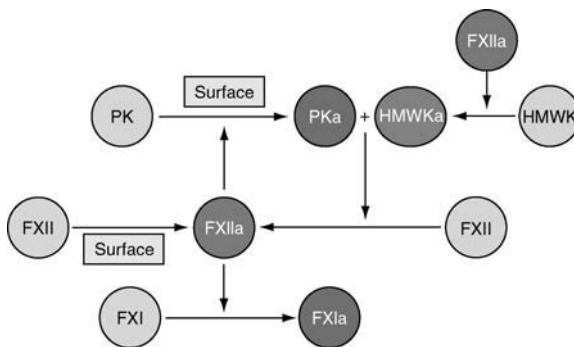
Without effective control mechanisms, the basic principle of self-enhancing amplification loops within the clotting cascade would lead to complete vessel occlusions (thrombosis) once a vessel wall defect occurs, or activated clotting factors would be transported from the side of a vessel injury with the blood stream to other areas causing unwanted clotting there. The anticoagulant mechanisms controlling the clotting process either inactivate clotting factors directly (e.g., FXa, FIIa), or inactivate cofactors (e.g., FVa).

Tissue Factor Pathway Inhibitor

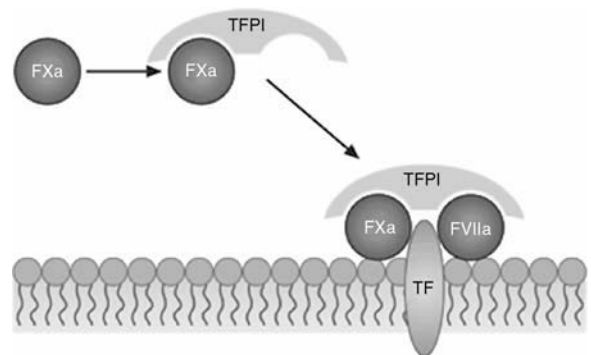
Primarily, tissue factor pathway inhibitor (TFPI) binds to and inactivates FXa. In a second step TFPI/



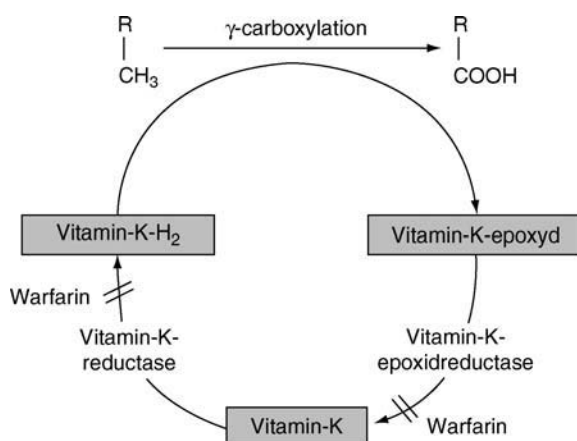
Coagulation/Thrombosis. Figure 4 Coagulation/Thrombosis.



Coagulation/Thrombosis. Figure 5 Coagulation/Thrombosis.



Coagulation/Thrombosis. Figure 7 Coagulation/Thrombosis.

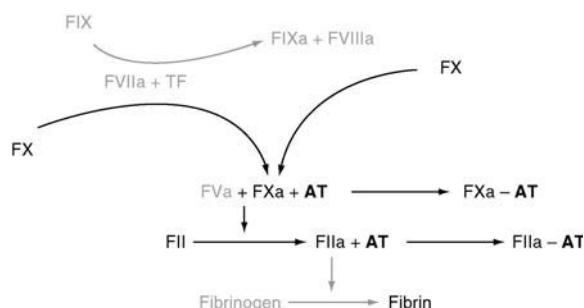


Coagulation/Thrombosis. Figure 6 Coagulation/Thrombosis.

FXa complexes bind to and neutralize tissue factor/FVIIa complexes, the key starting point of the extrinsic clotting cascade (see earlier) (Fig. 7). Heparin is able to enhance this reaction by direct binding to the complex and by releasing TFPI from the unaltered vessel wall, which then can access the TF-exposing surface.

Antithrombin-Heparan Sulfate/Heparin System

Antithrombin (AT) is synthesized by the liver. It forms 1:1 complexes with FXa and FIIa (Fig. 8). Although AT binds to the active centre of the clotting factors FXa and FIIa (thrombin) it is not degraded by these proteases, but forms covalently linked complexes (pseudosubstrate). These AT-clotting factor complexes are degraded in the reticuloendothelial system. Under physiological conditions, AT is catalyzed by endothelial-cell-surface-bound heparansulfate, which binds to AT.



Coagulation/Thrombosis. Figure 8 Coagulation/Thrombosis.

Catalyzation of AT is the major principle of anticoagulatory treatment with heparin. The length of high molecular weight heparins (>18 glucose units) allows formation of a trimolecular complex of AT and FIIa (thrombin) or FXa in which heparin is binding to both molecules, catalyzing thrombin inactivation. Smaller heparin molecules (low molecular weight heparins or the even smaller pentasaccharide) bind only to AT. This still enables inactivation of FXa but no longer inactivation of thrombin.

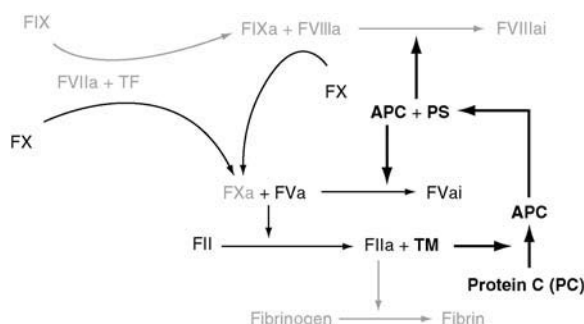
Besides AT, heparin cofactor II (HCII) is an anticoagulatory protein enhanced by heparin. HCII inactivates thrombin and the nonclotting enzymes cathepsin-G and chymotrypsin.

Whereas patients with AT deficiency present clinically with a high risk for thrombosis, HCII-deficient patients do not.

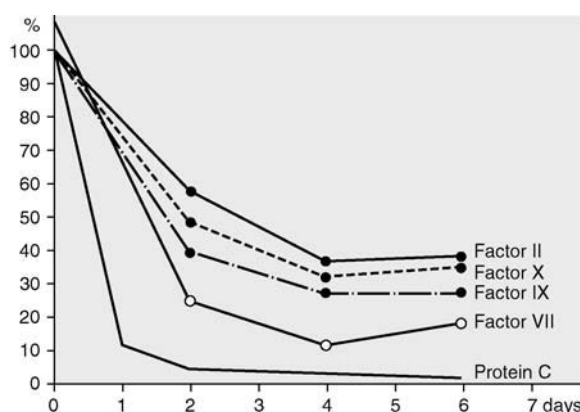
Protein-C Pathway

The protein-C pathway is one of the most important anticoagulant mechanisms. It is activated by thrombin. Thrombin binds to a cofactor in the membrane of endothelial cells, thrombomodulin (TM). TM bound thrombin no longer activates clotting factors or platelets but becomes an effective protein C (PC) activator. Activated PC (APC) forms a complex with Protein S, which inactivates FVIIIa and FVa. Hereby generation of FIIa by the prothrombinase complex is inhibited (Fig. 9). Thus, the PC-pathway controls thrombin generation in a negative feedback manner.

In about 30–40% of patients with suspected inherited thrombophilia the PC-pathway is disturbed by a mutation of FV (FV-Leiden). The FV-Leiden mutation affects one of the APC cleavage sites within the FV molecule. As a consequence, mutated FVa becomes resistant to rapid APC inactivation (APC resistance). About 4–7% of the middle European population carry this polymorphism of FV. Inborn deficiencies of Protein-S or Protein-C are much less frequent (< 1% and 0.2–0.4%, respectively).



Coagulation/Thrombosis. Figure 9 Coagulation/Thrombosis.



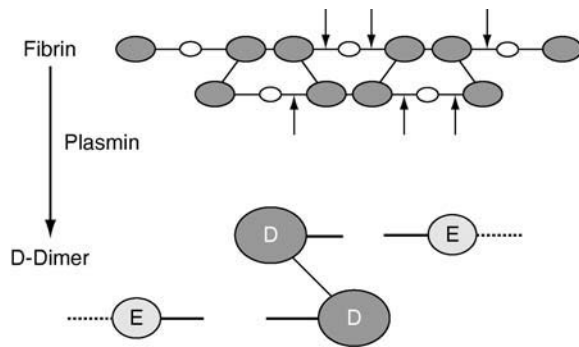
Coagulation/Thrombosis. Figure 10 Coagulation/Thrombosis.

Iatrogenic PC deficiency always occurs at the beginning of oral anticoagulation with vitamin K-antagonists. Due to the short half-life of PC, its plasma concentrations decline within one day, whereas the procoagulatory clotting factors are still present in high concentrations. This makes it mandatory to start vitamin K-antagonist treatment only under parallel parenteral anticoagulation with heparin for about 5 days (Fig. 10).

A new aspect of the PC-pathway is the efficacy of recombinant-APC in reducing mortality in patients with septic shock. Whether this is related to the inhibition of thrombin generation or due to other biological activities of APC is currently under investigation.

Fibrinolysis

Lysis of clots is another system for regulation of the clotting system. Plasmin is the key enzyme of the fibrinolytic system. Its proenzyme plasminogen is synthesized by the liver as a one-chain protein. Tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) activate plasminogen by cleavage. The remaining two chains are linked by



Coagulation/Thrombosis. Figure 11 Coagulation/Thrombosis.

disulfide bonds and undergo autocatalytic cleavage at the N-terminus; thus, plasmin self-enhances its fibrinolytic activity.

Plasmin cleaves fibrin at different positions. Of high clinically practical relevance is the cleavage of the fibrin γ -chain that results in D-Dimers (Fig. 11). D-Dimers are specific for fibrin cleavage by plasmin. They can easily be detected by commercially available assays and are used to exclude thrombosis. A negative test for D-Dimer has a high negative predictive value for a thrombosis.

Therapeutically t-PA and urokinase are the most important drugs for fibrinolytic therapy (myocardial infarction, stroke, massive pulmonary embolism). This treatment is associated with an enhanced risk of bleeding complications.

Regulation of the Fibrinolytic System

Generation of plasmin is inhibited by the plasmin-activator inhibitor (PAI). PAI is secreted by endothelial cells. It neutralizes t-PA and u-PA by forming 1:1 complexes.

Nonfibrin bound, free plasmin is inactivated by α_2 -anti-plasmin by an irreversible, covalent 1:1 complex.

- Anticoagulants
- Antiplatelet Drugs
- Fibrinolytics

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Coatomer

Coatomer is the major coat component of COPI vesicles. The Coatomer complex consists of seven different subunits (α - ζ COP).

- Intracellular Transport

Cocaine- and Amphetamine-regulated Transcript (CART)

- Appetite Control
- Psychostimulants

Co-chaperone

A nonsubstrate protein that interacts specifically with a molecular chaperone, and is important for efficient chaperone function.

- Chaperones

Co-dominant

A co-dominant is a heritable trait in which both alleles of a polymorphism are expressed and are reflected in the phenotype. The phenotype of heterozygous carriers is in between the phenotypes of the two homozygous genotypes.

- Pharmacogenetics

Coenzyme Q₁₀

Coenzyme Q₁₀ (ubiquinone) is a coenzyme in the mitochondrial respiratory chain. It has a side chain made up of 10 isoprene units. Its synthesis can be inhibited by

HMG-CoA reductase inhibitors (statins). This effect has been suggested to account for some of the side effects of statins like myositis or rhabdomyolysis.

► HMG-CoA Reductase Inhibitors

Congenital Nephrogenic Diabetes Insipidus

Synonyms

NDI

Definition

Disorder characterized by an inability to concentrate urine in response to vasopressin due to mutations in the vasopressin V2 receptor gene or the AQP2 gene.

- Aquaporins
- Vasopressin/Oxytocin

Collagen

Collagen is a major component of connective tissue that becomes exposed at the subendothelium of injured blood vessels. It contributes to platelet adhesion and also plays a role in platelet activation by binding to several receptors on platelets such as integrin $\alpha 2\beta 1$ or glycoprotein VI (GP VI).

- Antiplatelet Drugs
- Matrix Metalloproteinases

Colony-stimulating Factors

Colony-stimulating factors (CSFs) belong to the group of cytokines, and function as haematopoietic factors [e.g. GM-CSF (filgrastim, lenograstim), GM-CSF (molgramostim, sargramostim)]. Colony-stimulating factors stimulate the growth and differentiation of haematopoietic progenitor cells.

- Hematopoietic Growth Factors
- Cytokines

Combinatorial Chemistry

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Definition

Combinatorial chemistry constitutes a branch of the molecular sciences, providing an array of concepts and methods to solve molecular optimization problems (in drug research and beyond) more rapidly and efficiently than classical synthetic approaches.

Description

Combinatorial chemistry provides an array of concepts and methods to solve molecular optimization problems via the use of chemical libraries. Combinatorial chemistry mimics evolution cycles of synthesis and selection in the chemical laboratory. Since specific molecular interactions between proteins and their ligands have been recognized as the molecular basis of most biological processes including disease, it became possible to study and optimize the interactions between drugs and their target proteins on a molecular level. Thus, drug development has turned into a systematic and rational task of optimization. During the 1990s in the pharmaceutical industry, synthetic chemistry became evident as the major bottleneck in drug development. Combinatorial chemistry was the answer of the synthetic chemistry community. The term “combinatorial” is derived from the mathematical discipline combinatorics dealing with the statistics of element combinations. Combinatorics are employed in chemistry to calculate the number of possible combinations of m chemical building blocks in n synthetic cycles (n^m , see Fig. 1). For example, the 20 native amino acids can be combined to form 20^6 different linear hexapeptides.

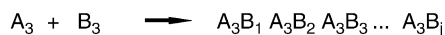
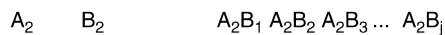
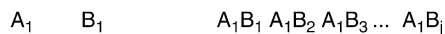
Centerpieces of combinatorial concepts include the synthesis of compound libraries instead of the preparation of single target compounds. Library synthesis is supplemented by approaches to optimize the diversity of a compound collection (diversity-oriented synthesis) and by efforts to create powerful interfaces between combinatorial synthesis and bioassays.

These conceptual goals are attained by several combinatorial methods and tools. Characteristic for combinatorial chemistry is the synthesis on solid support or by polymer-supported synthesis, allowing for much higher efficiency in library production. Synthesis can be conducted either in automated parallel synthesis or by ► [split-and-recombine synthesis](#). Centerpieces of combinatorial methods further include specific analytical methods for combinatorial

Classical synthesis



Combinatorial chemistry



Building blocks (Starting materials)	Combinatorial library (Products)
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Combinatorial Chemistry. Figure 1 Whereas in classical chemical synthesis one target molecule was prepared in combinatorial chemistry the systematic combination of building blocks generates chemical libraries.

chemistry and computer-aided methods for combinatorial chemistry.

Chemical Libraries

Precisely defined collections of different chemical compounds are denominated as chemical libraries that can be efficiently prepared by methods of combinatorial chemistry. Each chemical compound owes specific structural, steric, and electronic properties that determine all possible interactions of the small molecule with a given protein or receptor. The molecule's properties are based on the steric arrangement of functional groups, including the conformations that can be attained by a specific structure.

Complex optimization of the ligand-protein interactions require to scan large areas of the chemical space. Thus, the combinatorial chemist aims not at the preparation of single compounds but of chemical libraries. Chemical libraries can be produced as collections of single compounds or as defined mixtures.

Diversity-Oriented Synthesis and the Chemical Space

The challenge in the synthesis of chemical libraries is the vast number of different, potentially drug-like small molecules which is estimated to be as high as 10^{60} . As all of these molecules can never be synthesized and tested, it is essential to define criteria for the composition of libraries spanning the biologically relevant areas of the chemical space most efficiently. An important criterion of a compound library is its chemical diversity, a term describing the similarity or dissimilarity of all library components. Thus, chemical diversity expresses how well a library represents all theoretical possibilities within the chemical property space. A library with low

chemical diversity contains molecules that are relatively similar, thus covering a small area of the accessible property space. On the contrary, a library with a large chemical diversity will contain relatively dissimilar molecules, covering a large volume of the property space. The diversity of a library is a major criterion of its quality and its possible applications. Large diversity is required mainly in the early stages of drug development (lead search), whereas defined small diversity (focussed libraries) is needed for lead optimization. Contrary to classical organic synthesis which was directed at the preparation of a single target compound, diversity-oriented synthesis aims at providing libraries with defined chemical diversity, with the help of combinatorial methods and tools described herein.

Solid Phase Synthesis Versus Polymer-Supported Synthesis in Solution

The evolution of combinatorial methods was closely linked to the development of polymer-supported synthesis, which opened efficient access to diversity-oriented synthesis. In ►solid phase synthesis, forming the backbone of most combinatorial methods, insoluble organic or inorganic matrices are employed as carriers for the construction of product molecules. Using insoluble polymer supports is advantageous, strongly facilitating the isolation of intermediates and products attached to the polymer support. By-products formed in each stage of the synthesis are easily removed by washing the polymer support, thus each isolation step is reduced to simple filtration. By employing reagents in high excess, complete conversion can be attained on the solid phase in reactions that form mixtures in solution. The relative isolation of reactive sites on the polymer supports can be exploited for specific synthetic effects such as favoring cyclization and the reduction of by-products. Finally, the compartmentalized structure of polymer supports allows for split-and-recombine synthesis.

Under certain condition, however, reactions are still preferably conducted in solution. This is the case e.g., for heterogeneous reactions and for conversions, which deliver complex product mixtures. In the latter case, further conversion of this mixture on the solid support is not desirable. In these instances, the combination of solution chemistry with polymer-assisted conversions can be an advantageous solution. Polymer-assisted synthesis in solution employs the polymer matrix either as a scavenger or for polymeric reagents. In both cases the virtues of solution phase and solid supported chemistry are ideally combined allowing for the preparation of pure products by filtration of the reactive resin. If several reactive polymers are used sequentially, multi-step syntheses can be conducted in a polymer-supported manner in solution as well. As a further advantage, many reactive polymers can be recycled for multiple use.

Parallel Synthesis and Automation

If small or medium libraries for lead optimization are demanded and all synthetic products are to be screened individually, most often parallel synthesis is the method of choice. Parallel syntheses can be conducted in solution, on solid phase, with polymer-assisted solution phase syntheses or with a combination of several of these methods. Preferably, parallel syntheses are automated, either employing integrated synthesis robots or by automation of single steps such as washing, isolation, or identification. The latter concept often allows a more flexible and less expensive automation of parallel synthesis.

Split-and-Recombine Synthesis for the Preparation of Large Libraries

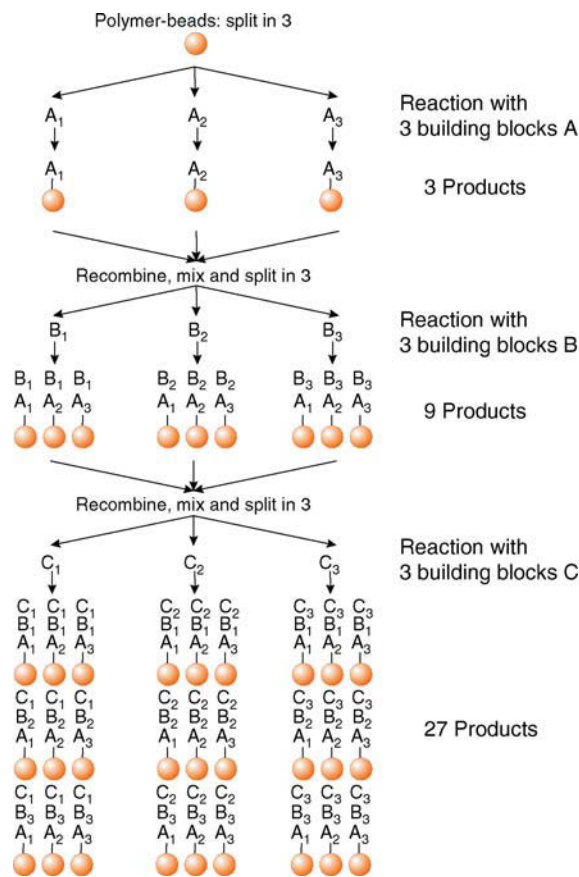
If large or very large libraries are demanded and if a powerful interface to a subsequent bioassay is available, split-and-recombine synthesis can be the method of choice. Split-and-recombine synthesis is mostly conducted on solid phase. During each coupling step the resin is divided into one reactor for each coupled building block (split). Following the coupling reaction with m building blocks, all resin beads are pooled and mixed (recombine) (see Fig. 1). Consequently, after n coupling steps m^n different compounds are obtained in theory, the real number of individual compounds is only limited by the number of available resin beads. As in each coupling step only one building block is coupled per reaction vessel, in the end on every bead only one compound is produced (one bead-one compound) (Fig. 2).

Introduced in the early 1990s, the split-and-recombine concept contributed much to the early success of combinatorial chemistry. Often, all combinatorial methods were identified with this concept. Split-and-recombine synthesis offered easy access to large number of individual compounds in few steps. If conducted on polymer beads, these are easily separated mechanically and can be identified subsequent to a screening step.

Though split-and-recombine techniques make available many compounds in few steps, the concept possesses significant limitations as well. A well-defined synthesis requires employing reliable reactions for all the different starting materials. This requirement is fulfilled only for few reaction types, such as acylations of amines, reductive aminations, substitution of α -halogeno carbonyl compounds. If however, the products of a split-and-recombine synthesis are not obtained purely, it becomes increasingly difficult to ascribe a biological signal unambiguously to an individual compound.

Analytical Methods in Combinatorial Chemistry

Essential prerequisites for the evolution of combinatorial methods were the progress in reaction monitoring and analytics. Of specific importance was the analytics



Combinatorial Chemistry. Figure 2 Chemical libraries are prepared either by parallel synthesis or by the split-and-recombine method. In the latter case, coupling m building blocks in m separated reaction flasks through n synthetic cycles on a beaded polymer carrier generates a combinatorial library with m^n individual compounds and one compound per bead.

of structures attached to a polymeric support (► [on-resin-analysis](#)) as well as the analytics of cleaved compounds in solution (off-resin-analysis).

For on-bead analysis vibrational spectroscopy (IR-spectroscopy) can be employed; attenuated total reflection is a method allowing fast and nondestructive on-bead analysis of small samples (single bead analysis) without significant sample preparation. Solid phase ► [NMR](#) is the method of choice if complex structural analysis is intended on the support. Spatially resolved analysis on the resin is possible with microscopic techniques.

For off-bead analysis, coupling between chromatographic separation and mass spectrometric detection has proven especially powerful. The combination between high performance liquid chromatography (HPLC) and ► [electrospray ionisation mass spectrometry](#) has the advantage that purity of product mixtures can be coupled on-line with the product identification.

Integration of Combinatorial Synthesis and Screening

The potency of combinatorial chemistry is optimally exploited by efficiently integrating combinatorial synthesis with a subsequent bioassay. Chemical libraries are mostly screened in parallel as on microtiter volumes, on spatially addressable molecular arrays, or as complex mixtures as in the case of on-bead assays. The latter approach is especially yielding, as large libraries of synthetic compounds can be screened simultaneously. If the synthetic chemistry is controlled well, it becomes possible through the integration of synthesis and screening to first select the most active compounds, analyze, and resynthesize them for verification. By this method the enormous effort necessary for the parallel screening of large libraries is significantly reduced. This concept was especially successful for the on-bead screening of split-and-recombine libraries. Hits (i.e., the most active compounds) are preferably visualized by fluorescence or color, can be separated by manual or automated selection and subsequently analyzed. Identification of hits is effected by noncovalent binding of fluorophor-labeled proteins, or in the case of polymer-based protease inhibitor assays, by a fluorescent dye that is formed dependent on the inhibitor activity. Identification of hit structures is facilitated by tagging of the beads with easily detectable structures that code for the synthesized molecule on one individual bead.

Bioinformatics and Combinatorial Chemistry

One reason for the initial popularity of combinatorial methods was the dissatisfaction with and the limited success of rational molecular design in the 1980s. Meanwhile, it has been recognized that combinatorial chemistry can profit strongly from computer-aided methods. It became obvious that all possible combinations of building blocks cannot be prepared synthetically due to limited physical resources and for cost reasons. Bioinformatic methods today are used to describe and analyze the diversity of chemical libraries. Moreover, aggregated biological data of chemical compounds are used to determine quantitative structure-activity relationships (QSAR) that deliver criteria for the composition of screening libraries and for systematic hit variation. A prominent example is the Lipinski rules summarizing molecular descriptors to be obeyed by orally available drugs.

If structural information of the protein target is available, e.g., a crystal structure, in silico screening of huge virtual compound libraries can be conducted by the use of docking simulations. Based on identified primary hits, structural variations of the ligand can be evaluated by computational modeling of the ligand-protein complex.

Pharmacological Relevance

Combinatorial chemistry is the branch of the molecular sciences providing concepts and methods for solving problems of molecular optimization fast and efficiently.

Since drug development has turned into a systematic and rational task of optimizing molecules and their interactions with proteins, cells, and organisms, combinatorial chemistry has become a significant part of this endeavor. Combinatorial methods are mainly employed in the initial (preclinical) stages of drug development.

- High-throughput Screening
- NMR-based Ligand Screening
- Bioinformatics

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Compartment

A compartment is an anatomical space in the body into which a drug or metabolite, or a chemical derivative or metabolite formed from the parent drug may distribute.

- Pharmacokinetics

Competitive Antagonists

By definition, competitive antagonists compete with the agonist for the same binding domain on the receptor. Therefore, the relative affinities and the relative concentrations of the agonist and antagonist dictate which ligand dominates. Under these circumstances, the concentration of agonist can always be raised to the point where the concomitant receptor occupancy by the antagonist is insignificant. When this occurs, the maximal response to the agonist is observed, i.e., surmountable antagonism results.

- Drug-Receptor Interaction

Complement System

A cascade of proteins of the immune response that can be triggered by antigen-antibody complexes and by the innate immune system (e.g. exposure to microbial polysaccharides) to raise the immune response. Complement proteins can detect and bind to foreign material or immune complexes and label them for phagocytosis. They can also cause inflammation by directly degranulating mast cells and releasing chemokines to recruit other immune cells into the affected area.

- ▶ Immune Defense
- ▶ Inflammation
- ▶ Histaminergic System

Complement-type Repeat

Forty-four amino acid module characterized by three internal disulfide bridges and an octahedral cage for a calcium ion. Complement-type repeats are found in many cell surface proteins and form the ligand-binding domain of receptors of the LDL receptor gene family.

- ▶ Low-density Lipoprotein Receptor Gene Family

Complex Disease

A disease whose pattern of familial aggregation differs from that expected from the Mendelian inheritance of a single genetic defect.

- ▶ Pharmacogenomics

Compound Libraries

A compound library is a compound collection. The compound libraries of large pharmaceutical and screening companies can exceed 1 million samples. Libraries are synthesized and stored as either individual samples or as combinations.

- ▶ Combinatorial Chemistry
- ▶ High-throughput Screening

Compound Optimization

Compound optimization in early- and late-phase drug discovery is covered, emphasizing physicochemical properties, in vitro absorption, metabolism and in vivo animal pharmacokinetic methodologies.

- ▶ Combinatorial Chemistry
- ▶ Gene Expression Analysis

Computational Biology

- ▶ Bioinformatics

Computerized Tomography

Slices of the body are irradiated from one side. X-ray detectors quantify the remaining intensity of the X-rays after passing through the various tissues. The X-ray tube and the detectors are rotating around the body. The data are used to calculate images of the corresponding slice which reflect the absorption of X-rays in a great number of pixels of an individual slice.

- ▶ Radiocontrast Agents

COMT

- ▶ Catechol-O-Methyl-Transferase

Conditioned Place Preference

The conditioned place preference paradigm is widely used in order to measure the rewarding properties of drugs of abuse (secondary reinforcement). In general individuals are subsequently injected with either the drug or vehicle solution and placed into boxes with

floors with different textures (i.e., either perforated or bar). In this way, the association between the administration of the drug and a particular floor texture (or place) is established. On the final day of the experiment, individuals are left untreated and placed in conditioning boxes that have the two different floor types. To determine the rewarding effects of the drug, the time that each individual stays on the floor type paired with the drug is measured. If the drug has rewarding properties the individual shows a preference to the side that has been paired with drug administration.

► Drug Addiction/Dependence

Conditioned Withdrawal

Individuals experiencing drug withdrawal can become conditioned to environmental situations. Previously neutral stimuli can elicit many of the symptoms of drug withdrawal, and this “conditioned withdrawal” has motivational significance especially in alcohol and opiate addiction. Thus conditioned withdrawal may trigger ►craving and relapse in a particular situation.

► Drug Addiction/Dependence

Conditioning

Mechanism through which repeated associations between two stimuli induce a new learned response. In particular, by pairing a neutral stimulus (conditioned stimulus) with an unconditioned stimulus (that induces a physiological response) many times, the neutral stimulus alone will be capable of producing a conditioned physiological response.

► Placebo Effect

Congenital Long QT Syndrome

A disease predisposing those affected to severe cardiac arrhythmia. The term “long QT syndrome” refers to an abnormality found in the electrocardiograms of the patients: a long QT interval caused by a prolonged

repolarisation period of the cardiac myocytes. The disease-causing mutations are located in genes encoding the various subunits of voltage-gated K^+ channels involved in depolarization and repolarization of cardiac myocytes. An example are mutations in the human-ether-a-go-go-related protein (HERG).

► Protein Trafficking and Quality Control
► Voltage-gated K^+ Channels

Conjugative Plasmids

Plasmids are termed conjugative they have the capacity to transfer themselves from one cell to the other. They can be either self-transmissible or mobilizable (the latter necessitate the presence of a self-transmissible plasmid that supplies missing transfer functions). The *tra* genes encode among other proteins the subunits that form a sex pilus required to mediate the first contact between the donor and the recipient cell (in case of plasmids of gram-negative bacteria). Both cells then come into close contact and form a stable mating pair. The plasmid DNA in the donor cell is then cleaved at an *oriT* site (origin of transfer), unwound and one strand is transferred into the recipient cell. Complementary DNA strands are synthesized and recircularized.

► Microbial Resistance to Drugs

Conjugative Transposon

Conjugative transposons are self-transmissible large DNA elements (up to 150kbp) located in the donor chromosome. After excision a circular intermediate is formed that is unable to replicate autonomously. It is nicked at a origin of transfer site and one strand is then transferred to the recipient cell. After generating a double-stranded circle the transposon integrates into the recipients chromosome.

► Microbial Resistance to Drugs

Conotoxins

Conotoxins are the venoms of the marine cone snails. The >500 *Conus* species produce >10,000 different toxins. All are cysteine-rich peptides of 10–30 amino

acids. Many act on their target molecules with high selectivity. The α -conotoxins are competitive antagonists at nicotinic acetylcholine receptors. The μ -toxins block voltage-sensitive Na^+ channels. The ω -toxins block Ca^{2+} channels. Synthetic ω -conotoxin MVIIA (ziconotide) has been introduced into therapy for spinal administration in chronic pain. Like morphine (but by direct channel blockade rather than through a G protein pathway) it inhibits transmitter release from nociceptive afferents.

- Synaptic Transmission
- Voltage-dependent Na^+ Channels
- Voltage dependent Ca^{2+} Channels
- Nicotinic Receptors

Contact Inhibition

Contact inhibition is observed in the process of wound healing and describes the ability of a tissue to stop cell proliferation again after cellular multiplication has filled up the defect caused by a wound.

- Antineoplastic Agents

Contraceptives

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Synonyms

Hormonal contraceptives; Oral contraceptives

Definition

Hormonal contraceptives belong to the most widely prescribed and most efficacious drugs that have a profound impact on western societies since their inauguration in the 1960s. In women, oral hormonal contraceptives are used to prevent fertilization or implantation in cases of unplanned pregnancies. Apart from these primary objectives, there are significant additional medical benefits contributing to a substantial improvement of reproductive health in women.

Mechanism of Action

The most frequently used oral contraceptives are composed of varying combinations of ►estrogens and

►progestins, which belong to the large family of ►steroid hormones. Steroids interact with intracellular receptors functioning as ligand-activated transcription factors to control the expression of a wide array of specific genes. The receptors for estrogens and progestins are members of a superfamily of approximately 150 structurally related nuclear receptors that bind ligands such as steroid hormones, retinoids, vitamin D_3 , eicosanoids, and thyroid hormones [1]. Members of this receptor superfamily share a common architecture of mainly four conserved functional domains: The N-terminal transactivation domain (activation function, AF-1) is the most variable in the superfamily of receptors. The subsequent DNA-binding domain also participates in receptor dimerization, whereas nuclear localization is determined by the neighbouring hinge region. The C-terminal hormone-binding domain comprises a ligand-dependent transcriptional activation function (AF-2) and provides the sites for the binding of chaperones like heat shock proteins that prevent dimerization and DNA binding of unliganded receptors.

Upon hormone binding, steroid hormone receptors undergo a conformational change, dissociate from heat shock proteins and translocate into the nucleus where they interact as dimers with specific DNA regulatory sequences of target genes. The transcriptional regulation by steroid hormone receptors is mediated by co-regulatory proteins that either positively (►co-activators) or negatively (►co-repressors) influence steroid hormone-induced transcriptional activity. A complementary mode of steroid hormone receptor action relies on direct protein-protein interactions with other transcription factors such as AP-1, Sp-1, and NF- κ B, thus providing an explanation for the well-known observation that steroid hormone receptors are able to regulate genes lacking consensus response elements in their non-coding 5' region [2].

Most nuclear receptors are phosphoproteins, and their function can be influenced by phosphorylation events that are initiated by membranous receptors like receptor tyrosine kinases or G-protein-coupled receptors. Thus, steroid hormone receptors are embedded in complex signalling networks that may give rise to ligand-independent activation of nuclear receptors. In addition to the well-understood nuclear events set in motion by steroid hormones, rapid non-genomic effects have been reported for estrogens and progesterone. These rapid effects of sex-steroids that are often initiated at the plasma membrane, may result from receptor-independent alterations of plasma membrane fluidity or from steroid effects on membranous receptors other than classical steroid hormone receptors. In particular, the rapid engagement of the MAP kinase cascade by estrogen has been studied in great detail, and it is quite likely that cardinal estrogenic effects like cell proliferation and survival are not primarily brought about by

genomic actions of classical nuclear receptors but by rapid, non-genomic mechanisms [3].

At present, two distinct nuclear estrogen receptors, ER α and ER β , are known. The receptors differ in their tissue distribution, ligand binding profile and transcription activation functions in that ER β is devoid of AF-1. In cells, that express both estrogen receptors, ER β appears to oppose the transcriptional activity of ER α . Due to two distinct estrogen-dependent promoters in the single progesterone receptor (PR) gene, two isoforms of the progesterone receptor, PR-B and the N-terminally shortened PR-A, are generated. The biological activities of PR-A and PR-B are distinct and depend on the target tissue in question. In the reproductive system, progesterone acting through its respective nuclear receptors, PR-A and PR-B, serves the role of a physiological negative regulator of estrogen action by causing depletion of estrogen receptors. In addition, the major role of PR-A may be to inhibit transcriptional activity of other steroid receptors like ER α . Although one aspect of the biological relationship between estrogen and progesterone may be called functional antagonism, a concerted, sequential action of estrogen and progesterone is required in reproductive tissues like the endometrium and the breast to yield the desired complex biological response.

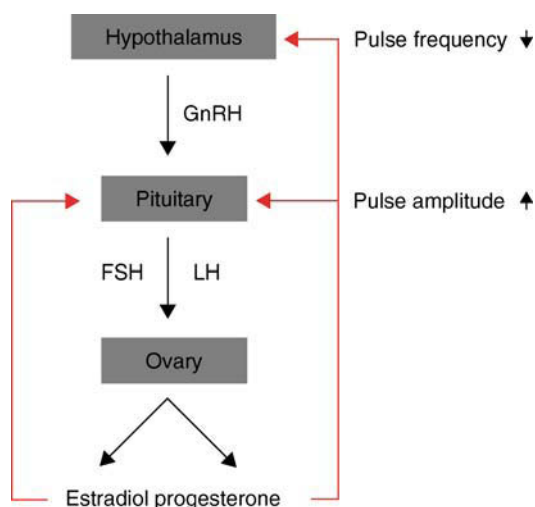
Estrogen and progesterone play a central role in the neuroendocrine control of the female menstrual cycle. In the early follicular phase estrogen exerts an inhibitory effect on the pulsatile secretion of **gonadotropins** from the pituitary (Fig. 1). Thus, the gradual increase in the peripheral estradiol concentration during the follicular phase is accompanied by a reduced release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the gonadotropes. At midcycle, a different set of regulatory interactions becomes dominant. A sustained

elevation of estradiol (150–200 pg/ml for approximately 36 h) induces a positive feedback on the anterior pituitary to trigger the ovulatory surge of LH. The underlying mechanism is a sensitization of the pituitary gonadotropes towards hypothalamic **gonadotropin-releasing hormone (GnRH)**. Progesterone decreases the frequency of GnRH release from the hypothalamus resulting in a marked decrease of the frequency of gonadotropin pulses in the luteal phase (Fig. 1). At the same time, progesterone increases the amount of LH released per pulse (i.e. the pulse amplitude).

Estrogen exerts a negative feedback on the pituitary and decreases the secretion of FSH and LH during most of the menstrual cycle. Yet, it triggers the LH surge at midcycle by sensitizing the pituitary to hypothalamic GnRH. Progesterone increases the amplitude of gonadotropins released from the pituitary and at the same time decreases the GnRH pulse frequency at the level of the hypothalamus. These feedback controls give rise to fairly frequent LH pulses of low amplitude during the follicular phase of the cycle and less frequent pulses with a higher amplitude in the luteal phase.

The main mechanism of action of a combination oral contraceptive (a combination oral contraceptive contains both an estrogenic and a progestational component to achieve contraception.) is to prevent ovulation by inhibiting gonadotropin secretion via an effect on both pituitary and hypothalamic centres. The progestational component primarily suppresses the surge-like LH release required to induce ovulation, while the estrogenic agent suppresses FSH secretion and thus prevents selection of a dominant follicle. Therefore, both estrogenic and progestational components of an oral contraceptive synergistically contribute to the contraceptive efficacy. However, even if follicular growth were not sufficiently inhibited, the progestational agent alone would suffice to abrogate the ovulatory LH surge. The estrogenic component, however, serves at least two other important purposes. It is responsible for the stability of the endometrium, thus minimizing events of irregular and unwanted breakthrough bleeding. In addition, estrogen action provides for a sufficient concentration of progesterone receptors. In aggregate, a small pharmacologic estrogen level is necessary to maintain the efficacy of the combination oral contraceptive.

As under most circumstances progesterone action will hold primacy over estrogenic effects, the cervical mucus, endometrium, and probably the fallopian tubes reflect progestational stimulation. The cervical mucus becomes thick and viscous and thus impervious to spermatozoa. The endometrium is in a state that is not receptive for implantation of a fertilized egg. Probably, the progestational impact on the secretory activity and peristalsis in the fallopian tubes also assists the general contraceptive effect. It is difficult, however, to assess the relative contribution of the various effects to the



Contraceptives. Figure 1 Regulation of hypothalamic and pituitary function by ovarian steroid hormones.

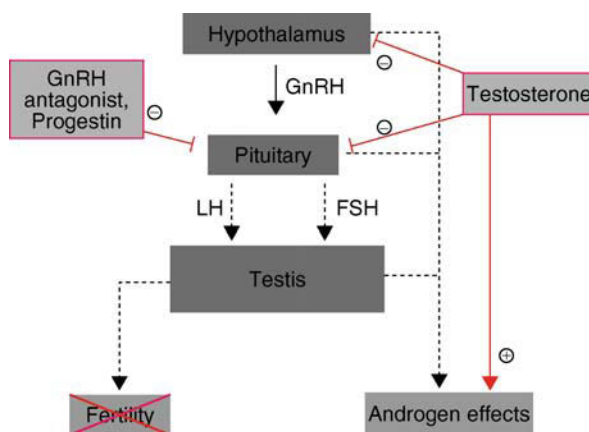
contraceptive efficacy, because combination oral contraceptives suppress ovulation very effectively.

The progestin-only minipill contains a low-dose of a progestational agent which is sufficient to block ovulation in only 60–80% of cycles. The contraceptive effect is largely dependent on endometrial and cervical mucus effects, as gonadotropins are not reliably suppressed. Because of the low-dose of progestins, the minipill must be taken every day at the same time with great accuracy.

Long-acting methods of hormonal contraception are even more effective than oral methods. Two effective systems are available: a sustained-release method of levonorgestrel or etonogestrel using implanted steroid-permeable silastic tubing and depot injections of medroxyprogesterone acetate. The mechanism of action is similar to the progestin-only minipill. However, in addition, the long-acting methods yield progestin plasma levels high enough to prevent ovulation in basically all patients.

High doses of estrogen to prevent implantation were used for emergency postcoital contraception. It was soon appreciated that the extremely large estrogen doses used (25–50 mg/day of diethylstilbestrol or ethinyl estradiol) entailed a high rate of gastrointestinal untoward effects. Clinical trials have ushered the use of combination oral contraceptives for emergency postcoital contraception. In principle, high-dose oral contraceptives can be administered within 72 h of intercourse, followed by a second dose, 12 h later. Such a regimen reduces the risk of pregnancy by approximately 75%. Multiple mechanisms appear to contribute to the treatment efficacy, for instance inhibition of ovulation, endometrial receptivity, cervical mucus composition, and tubular transport of spermatozoa. Alternatively, a high-dose of the progestin levonorgestrel (750 µg) can be used for postcoital emergency contraception. Administration of a total dose of 1,500 µg of levonorgestrel should be started within 12 h of unprotected sexual intercourse to achieve contraceptive protection of approximately 95%. Nausea, lower abdominal and breast pain as well as retarded menstruation have been reported as untoward effects. It is important to note that emergency contraceptives are not used as medical abortifacients to interrupt an established pregnancy defined to begin with implantation.

In recent years strategies for hormonal male contraception have also been devised. Quantitatively and qualitatively normal spermatogenesis critically depends on neatly orchestrated LH and FSH release from the pituitary. In hormonal approaches to male contraception gonadotropin secretion and intratesticular testosterone production needs to be suppressed as efficiently as possible. Because androgens are key determinants of virilization and thus the male phenotype, but also strongly suppress gonadotropin release when applied exogenously, androgen preparations are essential components



Contraceptives. Figure 2 Principles of hormonal male contraception. Testis function is regulated by hypothalamic and pituitary feedback circuits. The application of exogenous testosterone in conjunction with progestins or GnRH antagonists suppresses gonadotropin secretion from the pituitary and intratesticular testosterone production while maintaining extratesticular androgen effects. Such regimen reliably induced azoospermia and infertility.

of all experimental approaches to male contraception (Fig. 2). Notably, the use of long-acting testosterone esters such as testosterone undecanoate causes azoospermia in only 30% of Caucasian men, while androgen-only regimens are highly effective for East Asian men. As additional agents, various combinations of androgens with progestins or GnRH antagonists have been evaluated in clinical trials. Testosterone administered in combination with various progestins such as depot medroxyprogesterone acetate, norethisterone enanthate, desogestrel or etonogestrel has yielded promising efficacy.

Clinical Use (Including Side Effects)

After the seminal observation by Gregory Pincus and colleagues in the 1950s that progestins prevented ovulation in women, initial trials on humans were conducted using progestins like norethynodrel that were contaminated with about 1% mestranol. When subsequent efforts to provide a more pure progestin lowered the estrogenic component but provoked breakthrough bleeding, it was decided to keep the estrogen. Thus, the principles of combined estrogen–progestin oral contraception were established.

A major obstacle to the use of naturally occurring estrogens for the purpose of contraception was extensive first-pass hepatic metabolism and hence inactivation of the compounds when given orally. The addition of an ethinyl group at the 17 position made estradiol orally active. Ethinyl estradiol is a potent oral estrogen and

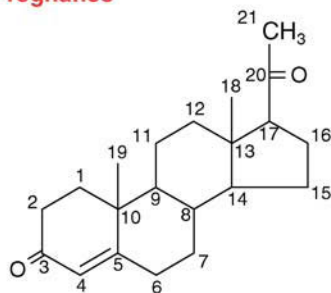
represents one of the two forms of estrogens used in oral contraceptive pills. The other estrogenic compound is the 3-methyl ether of ethinyl estradiol, mestranol which is converted to ethinyl estradiol in the body.

The progestins used in oral contraceptives are 19-nor compounds of the estrane and gonane series (Fig. 3). Each compound possesses various degrees of androgenic, estrogenic and antiandrogenic activities, thereby determining the scope of side effects. Animal and human studies showed, however, that only norethindrone, norethynodrel, and ethynodiol diacetate have estrogen activity. Replacement of the 13-methyl group of norethindrone with a 13-ethyl moiety gives rise to the gonane norgestrel, which is a potent progestin

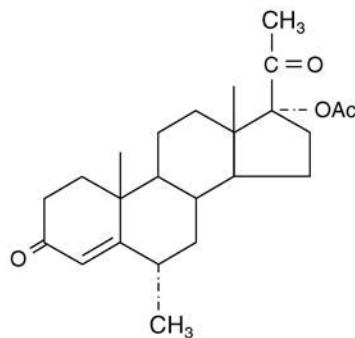
with reduced androgenic activity (Fig. 3). More recently developed compounds like desogestrel (Fig. 3), norgestimate and gestoden display the least androgenic characteristics when compared with other 19-nor substances. Although norgestimate is a “newer” progestin, its activity is believed to be largely mediated by levonorgestrel or related metabolites. Therefore, epidemiologists do not generally include combination contraceptives containing norgestimate in the group of third generation compounds.

In epidemiologic studies, all products containing less than 50 µg ethinyl estradiol per pill are summarized as ►[low-dose oral contraceptives](#). The first generation of oral contraceptives includes products with 50 µg

Pregnanes

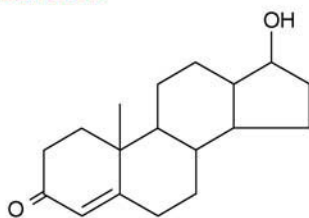


Progesterone

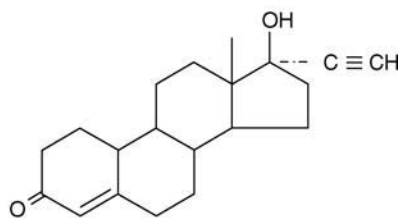


Medroxyprogesterone acetate

Estranes

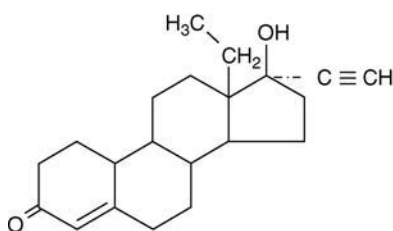


19-nortestosterone

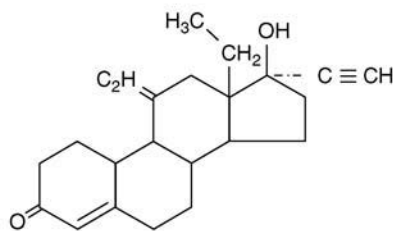


Norethindrone

Gonanes



Norgestrel



Desogestrel

Contraceptives. Figure 3 Examples of progestins derived from progesterone (pregnanes), 19-nortestosterone (estrane), and norgestrel (gonane).

or more of ethinyl estradiol. The second generation of oral contraceptives comprises formulations of norgestrel (0.3–0.5 mg), levonorgestrel (0.1–0.15 mg), norgestimate (0.25 mg), ethynodiol diacetate (1 mg), and other members of the norethindrone family in conjunction with 30 or 35 µg ethinyl estradiol. Desogestrel (0.15 mg) or gestodene (0.075 mg) are progestins in third generation contraceptives that are combined with 20 or 30 µg ethinyl estradiol. Most notably, the first oral contraceptive available contained 150 µg mestranol and 10 mg norethynodrel. It is nowadays commonly believed that the formulations of third generation oral contraceptives are very close to the lowest hormone levels which can be used without sacrificing contraceptive efficacy.

Combination oral contraceptives are the most frequently used agents and are characterized by a high therapeutic efficacy. Carefully controlled clinical studies with highly motivated subjects achieve an annual failure rate of 0.1%. The typical use effectiveness, however, amounts to 97–98%. Combination oral contraceptives are used as monophasic, biphasic, triphasic, and sequential preparations. In ▶monophasic preparations (Fig. 4), a fixed estrogen/progestin combination is present in each pill which is administered daily for 21 consecutive days followed by a 7-day hormone-free period (usually the pills for the last 7 days of a 28-day pack contain only inert ingredients). In the bi- and triphasic preparations (Fig. 4) varying amounts and ratios of estrogen to

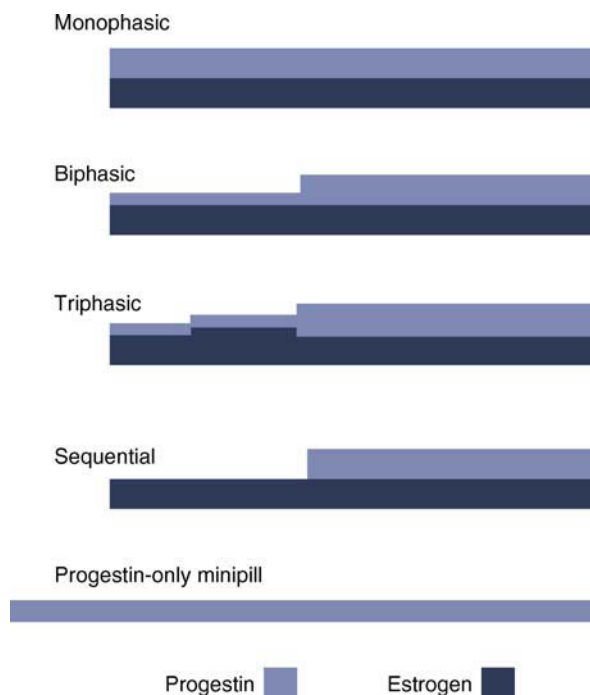
progestin are present in order to mimic most closely the sex-steroid levels throughout a normal menstrual cycle. In addition, the total amount of steroids administered can be reduced when taking multiphasic oral contraceptives. ▶Sequential preparations (Fig. 4) contain only estrogens for the first 7–11 days followed by a fixed estrogen/progestin combination in the remainder of the 21-day hormone application period. Phasic- and sequential-preparations were developed mainly to reduce the amount of progestins due to their untoward effects on the cardiovascular system.

Since a few years, a transdermal system received worldwide approval for hormonal contraception. In addition to ethinyl estradiol it contains norelgestromin as a progestational component. Norelgestromin is the active metabolite of norgestimate and is structurally related to 19-nortestosterone. Norelgestromin has negligible androgenic activity and may thus be suitable for women suffering from symptoms caused by androgen excess. The contraceptive efficacy of the transdermal patch is similar to that of oral contraceptives, but the transdermal system has the additional benefit of a once-weekly administration. In addition, transdermal delivery of hormones abolishes variability in gastrointestinal absorption and circumvents hepatic first-pass metabolism. It should be noted, however, that under steady-state conditions the area under the curve (AUC) describing plasma estrogen concentrations is 60% higher than corresponding levels resulting from a daily oral application of 35 µg ethinyl estradiol. Thus, a potential increase in cardiovascular complications cannot be ruled out with certainty so far.

Another fairly recent development in the field of hormonal contraception are combined contraceptive vaginal rings (NuvaRing) releasing 120 µg etonogestrel and 15 µg ethinyl estradiol daily. The small silastic ring is replaced weekly for 3 weeks followed by a ring-free period of 7 days. NuvaRing is well-tolerated and accepted by women, allows for a good control of the menstrual cycle and has a therapeutic efficacy comparable to oral contraceptives.

Progestin-only contraceptives (Fig. 4) contain low-doses of progestins (e.g. 350 µg norethindrone or 75 µg norgestrel) that have to be administered daily without interruption. The lowest expected failure rate during the first year of use is 0.5%, while the typical failure rate amounts to 3%. Subdermal implants of norgestrel (216 mg) for sustained release provides for long-term (for up to 5 years) contraceptive effects characterized by failure rates of only 0.05%. Reliable contraception for 3 months can be achieved by an intramuscular injection of a crystalline suspension of 150 mg medroxyprogesterone acetate (Fig. 3) (failure rate 0.3%).

The main purpose of healthy women taking oral contraceptives is to prevent unwanted pregnancies. “Primum non nocere” applies particularly to preventive



Contraceptives. Figure 4 Principal composition of various forms of oral contraceptives.

health care measures and therefore, untoward effects of oral contraceptives have to be monitored and assessed with great scrutiny. Shortly after the introduction of oral contraceptives approximately 40 years ago they soon became one of the most widely used drugs throughout the world. Hence, it is not surprising that reports on adverse effects began to appear rather quickly. Most of the untoward effects appeared to be dose-dependent, thus spurring on researchers to develop the current low-dose preparations. The most worrying adverse effects can be summarized in two main categories: the cardiovascular system and cancer.

Synthetic estrogens like ethinyl estradiol have a profound effect on the production of fibrinogen and clotting factors VII, VIII, X, and XII in the liver. In parallel, the concentrations of anticoagulation factors like protein C, S, and antithrombin III are diminished. This procoagulatory effect leads to an increased risk of thromboembolism in healthy women taking oral estrogens. In 1995, several studies reported on a twofold increase in the risk of venous thromboembolism when third generation oral contraceptives containing desogestrel and gestoden were compared with older preparations mostly containing levonorgestrel. This highly contentious issue was finally resolved when further analyses were able to explain the divergent results by confounding variables.

The effects of oral contraceptives on thrombosis can be surmised as follows: All low-dose oral contraceptives, regardless of the type of progestin, have an increased risk of venous thromboembolism. Smoking has no effect on the risk of venous thrombosis. However, smoking and estrogen administration have an additive effect on the risk of arterial thrombosis. Oral contraceptive-induced hypertension was previously observed in users of higher dose pills. An increased risk of clinically significant hypertension, however, has not been reported for low-dose oral contraceptives, including those containing the third generation progestins. Pre-existing hypertension is an important additive risk factor for stroke in oral contraceptive users. Most notably, recent clinical studies fail to find any substantial risk of myocardial infarction or stroke in healthy, non-smoking women, regardless of age, who take low-dose oral contraceptives. The vast majority of myocardial infarctions and strokes in oral contraceptive users occur when women over the age of 35 and cardiovascular risk factors take high-dose products (more than 50 µg ethinyl estradiol per pill). By meticulous screening for the presence of smoking and cardiovascular risk factors, especially hypertension, in older women, the risk of thromboembolic disease associated with low-dose oral contraceptives can virtually be annihilated.

In 1996, a metaanalysis of 54 epidemiologic studies indicated that women had a slightly increased risk of

breast cancer while taking oral contraceptives when compared to non-users (relative risk = 1.24). The increased risk diminished steadily after cessation of medication and was not found elevated 10 years after discontinuation. However, a recent population based case-control study of more than 4,500 women with breast cancer and nearly 4,700 controls showed no association between past and present use of oral contraceptives [4]. Due to the large study, subgroups of women, e.g. those taking a formulation with a high estrogen content, duration of oral contraceptive use, initiation of use during adolescence, history of breast cancer in a first-degree relative, could be analysed. None of these subgroups had a significantly increased risk of breast cancer. In light of these reassuring data, one has to conclude that oral contraceptive use is not associated with an increased risk of breast cancer. Such a conclusion contrasts sharply with the outcome of the Women's Health Initiative (WHI), the first randomised primary prevention trial on the effect of combined estrogen plus progestin (0.625 mg conjugated equine estrogen and 2.5 mg medroxyprogesterone acetate per day) on healthy postmenopausal women. The latter study was terminated early, chiefly because women receiving the active drug had an increased risk of breast cancer and the overall assessment was that the treatment was causing more harm than good [5]. It is therefore important to note that because of differences in doses and specific agents used, it is not justified to extrapolate adverse side effects of hormone replacement therapy to oral contraceptives or vice versa. In 2005, the WHO International Agency for Research on Cancer announced a reassessment of the carcinogenicity of combined estrogen-progestagen contraceptives and menopausal treatment. However, a meaningful discourse about this topic can only take place once the full complement of scientific data is available to the scientific community. Nevertheless, the announcement as such has already instigated vivid public discussions about the safety of hormonal contraceptives.

Thus, our attention should shift from the concern of potential adverse effects to the health benefits imparted by hormonal contraceptives. The use of oral contraceptives for at least 12 months reduces the risk of developing endometrial cancer by 50%. Furthermore, the risk of epithelial ovarian cancer in users of oral contraceptives is reduced by 40% compared with that on nonusers. This kind of protection is already seen after as little as 3–6 months of use. Oral contraceptives also decrease the incidence of ovarian cysts and fibrocystic breast disease. They reduce menstrual blood loss and thus the incidence of iron-deficiency anemia. A decreased incidence of pelvic inflammatory disease and ectopic pregnancies has been reported as well as an ameliorating effect on the clinical course of endometriosis.

Future efforts should be directed at optimizing current formulations to finally come up with an ideal oral contraceptive which would reduce the risk of breast, ovarian and endometrial cancer without any cardiovascular complications.

- Nuclear Receptors
- Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
- Selective Sex-steroid Receptor Modulators

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Contraction/Contracture

Contraction is a general term that refers to the mechanically activated state of myofibrils which is usually caused by action potential(s). Contracture means muscle shortening or tension development, which is not triggered by action potential(s), e.g. K^+ contracture, and caffeine or halothane contracture. The word is also used for deformity or distortion of fingers, hand or limb, such as Dupuytren's or Volkmann's contracture.

- Ryanodine Receptor

Contrast Media

- X-ray Contrast Agents and Molecular Imaging

Convulsant Drugs

Convulsant drugs are a group of drugs, which can induce seizures. These drugs include antagonists of glycine receptors (e.g. strychnine) and antagonist of the GABA_A-receptor (e.g. bicuculline, picrotoxin).

- Glycine Receptor
- GABA_A-receptor
- Seizures
- Antiepileptic Drugs

Convulsions

- Seizures
- Antiepileptic Drugs

Cooperativity Factor

An allosteric ligand has an effect on a receptor protein mediated through the binding of that ligand to the allosteric binding domain. The intensity of that effect, usually a change in the affinity of the receptor for other ligands or the efficacy of a ligand for the receptor, is quantified by the cooperativity factor. Denoted α , a positive value for α defines a potentiation, a fractional value an inhibition. Thus if $\alpha=0.1$, a maximal tenfold decrease in the affinity of a tracer ligand for the receptor would be produced by the allosteric modulator.

- Drug–Receptor Interaction

COPI Vesicle

COPI vesicles mediate anterograde transport from the intermediate compartment to the Golgi, transport within the Golgi apparatus and retrograde transport back from the Golgi to the ER by the recruitment of soluble

proteins from the cytoplasm, the GTP-binding protein ARF1 and the coat protein complex coatamer that lead to budding of COPI-coated vesicles.

► Intracellular Transport

COPII Vesicle

COPII vesicles are transport intermediates from the endoplasmic reticulum. The process is driven by recruitment of the soluble proteins that form the coat structure called COPII from the cytoplasm to the membrane.

► Intracellular Transport

Coregulators

Macromolecules that associate with nuclear receptors to modulate their transcriptional activity.

► Nuclear Receptors

Co-repressors

The antagonist-induced conformation of nuclear hormone receptors attracts co-repressors like Nco/SMRT (nuclear hormone receptor co-repressor/silencing mediator of retinoid and thyroid receptors) which further recruit other nuclear proteins with histone deacetylase activity. Their action leads to chromatin condensation, thus preventing the general transcription apparatus from binding to promoter regions.

► Nuclear Receptors

Cortico-medullary Solute Gradient

While the osmotic concentration of renal cortical tissue is isotonic, interstitial solute concentration begins to rise at the border between renal cortex and renal medulla to

reach maximum concentrations at the tip of the renal papilla. This rise in tissue osmolarity, the cortico-medullary osmotic gradient, is a consequence of the countercurrent multiplication along the loops of Henle driven by active NaCl retrieval across the water impermeable ascending limb. The interstitial solutes are about half NaCl and half urea. The medullary hypertonicity permits the generation of a hypertonic urine when the collecting duct epithelium is water permeable under the influence of vasopressin.

► Diuretics

Corticosteroids

► Gluco-mineralocorticoid Receptors

Corticosterone

A principle glucocorticoid produced in the zona glomerulosa of the adrenal cortex. It is involved in modulating stress responses, immune reactions and food metabolism.

► Gluco-mineralocorticoid Receptors

Corticotropin

Synonyms

Adrenocorticotrope Hormone (ACTH)

► Gluco-mineralocorticoid Receptors

Corticotropin Releasing Hormone

► CRH

► Gluco-mineralocorticoid Receptors

Co-stimulation

It is an essential step in the induction of adaptive immune responses. Successful T-cell activation requires multiple signals. One signal is provided by presentation of an antigen bound to cell surface MHC molecules on antigen-presenting cells to a specific T-cell receptor. In the absence of further signals, T cells become unresponsive and may ultimately be eliminated through apoptosis. An important co-stimulatory signal is provided by an interaction between members of the B-7 family (either CD80 or 86) on antigen-presenting cells and CD28 on T cells. Other key interactions between antigen-presenting cells and T cells are mediated by binding of ICAM-1 to LFA-1, CD40 to CD40 ligand, LFA-3 to CD2, and so on. After activation, T cells express CTLA-4 which interferes with the B-7–CD28 interaction and helps to return the cells to the quiescent state.

- ▶ Immune Defense
- ▶ T Cell Receptors
- ▶ Rheumatoid Arthritis

Costimulatory Molecules

- ▶ Co-stimulation

Cotransmission

Cotransmission is transmission through a single synapse by means of more than one transmitter. For example, to elicit vasoconstriction, postganglionic sympathetic neurones release their classical transmitter noradrenaline (which acts on smooth muscle α -adrenoceptors) as well as ATP (which acts on smooth muscle P2 receptors) and neuropeptide Y (which acts on smooth muscle Y₁ receptors).

- ▶ Synaptic Transmission
- ▶ Purinergic System
- ▶ Tachykinins and their Receptors

Cough

Cough is an essential protective reflex response to irritating stimuli in the respiratory tract. It involves the sudden, usually involuntary, expulsion of air from the lungs. It can prevent foreign bodies from entering the lungs, or aid the removal of mucus and irritants from the lungs.

- ▶ Antitussive Drugs

Coumarins

- ▶ Anticoagulants

COX-1

Cyclooxygenases I.

- ▶ Cyclooxygenases
- ▶ Non-steroidal Anti-inflammatory Drugs
- ▶ Antiplatelet Drugs

COX-2

Cyclooxygenases II.

- ▶ Cyclooxygenases
- ▶ Non-steroidal Anti-inflammatory Drugs

COXIBs

A class of drugs that effect their action by selectively inhibiting the activity of cyclooxygenase-2. They have potent analgesic, antipyretic, and anti-inflammatory

properties and have reduced gastrointestinal adverse side effects. An increase in cardiovascular side effects has been observed with the use of these drugs.

- ▶ Prostanoids
- ▶ Cyclooxygenases
- ▶ Non-steroidal Anti-inflammatory Drugs

CpG Dinucleotide Motif

CpG stands for cytosine phosphate guanine dinucleotide in a particular sequence context. CpG motifs are responsible for proliferative effects of antisense oligonucleotides, particularly with respect to B-lymphocytes. The optimal immune-stimulatory consensus sequence surrounding CpG is R1R2CGY1Y2, where R1 is a purine (mild preference for G), R2 is a purine or T (preference for A), and Y1 and Y2 are pyrimidines (preference for T).

- ▶ Antisense Oligonucleotides (ASON)
- ▶ DNA Vaccination and Genetic Vaccination

CRAC Channels

Calcium channels in the plasma membrane activated after receptor-mediated calcium release from intracellular stores. These channels are present in many cellular types and play pivotal roles in a multitude of cell functions. It was recently shown that Orai proteins are the pore-forming subunit of CRAC channels. They are activated by STIM proteins that sense the Ca^{2+} content of the endoplasmic reticulum.

- ▶ TRP Channels
- ▶ NFAT Family of Transcription Factors
- ▶ IP3 Receptors

Crack

Crack is the free base form of cocaine which in contrast to cocaine, the salt form, can be smoked. This inhalative application results in a very rapid, intense effect.

- ▶ Psychostimulants

Craving

There are opposing views in the field regarding the term “craving,” whether it describes a physiological, subjective, or behavioral state, if it is necessary at all to explain addictive behavior or is an epiphenomenon, which is not necessary for the production of continued drug use in addicts. The World Health Organization (WHO) agreed on the definition of craving as “the desire to experience the effect(s) of a previously experienced psychoactive substance.” Behavioral researches conceptualize craving within the framework of incentive motivational theories of behavior and modify the definition of craving as “incentive motivation to self-administer a psychoactive substance.” Such an operational definition of craving has the advantage of making the phenomenon of craving accessible to experimental investigation and making it measurable.

- ▶ Drug Addiction/Dependence

CREB

CREB stands for cyclic-AMP response element (CRE) binding protein and is a transcription factor. When phosphorylated by ▶cyclic AMP- and cyclic GMP-dependent Protein Kinases or other protein kinases it binds to gene promoters that contain a specific binding site. After binding, the respective transcription activity is modulated.

- ▶ Transcriptional Regulation
- ▶ Antidepressant Drugs

Cre/loxP

Cre is a bacterial recombinase (cre=causes recombination), which recognizes loxP sites of bacteriophage P. If two loxP (loxP=locus of x-ing over of bacteriophage P) sites have a parallel orientation, the DNA segment between these sites will be deleted by the action of the Cre recombinase.

- ▶ Transgenic Animal Models

CRH

CRH (Corticotropin releasing hormone) is expressed in the nucleus paraventricularis of the hypothalamus and drives the stress hormone system by activating synthesis and release of corticotropin at the pituitary and in turn corticosteroid from the adrenal cortex. CRH is also expressed at many other brain locations not involved in neuroendocrine regulation, e.g. the prefrontal cortex and the amygdala. Preclinical studies have shown that CRH also coordinates the behavioral adaptation to stress (e.g. anxiety, loss of appetite, decreased sleepiness, autonomic changes, loss of libido).

- ▶ Gluco-mineralocorticoid Receptors
- ▶ Antidepressant Drugs

Crk (Chicken Tumor Virus Regulator Kinase)

Adaptor protein, containing one SH2 and two SH3 domains, which assembles signaling complexes at receptors and focal adhesions.

- ▶ Adaptor Proteins

Cromones

Both disodium cromoglycate and nedocromil sodium have antitussive effects in humans. In this instance, their activity occurs by increasing the depolarisation of sensory nerves, which increases the threshold for an action potential and therefore inhibits the activity of these neurons.

- ▶ Antitussive Drugs
- ▶ Bronchial Asthma

Cross-packaging

Cross-packaging is the packaging of viral genomes into the virion shell of other viruses.

- ▶ Gene Therapy

Cross Talk

In general terms, cross talk refers to the interaction between signalling pathways, e.g. between pathways involving ▶ [heterotrimeric GTP-binding proteins](#) and ▶ [tyrosine kinase](#) pathways.

Another example is a recently discovered second mode of action by which nuclear receptors modulate transcription. In contrast to DNA-binding-dependent mechanisms, cross talk refers here to gene regulation by protein-protein-interaction of nuclear receptors with other transcription factors, such as AP-1 or NF- κ B. Consequently, the nuclear receptor acts as a corepressor or coactivator of transcription.

- ▶ Transmembrane Signalling
- ▶ Nuclear Receptors
- ▶ Gluco-mineralocorticoid Receptors

Cross Tolerance

Cross tolerance is a form of tolerance which may develop to the effects of pharmacologically related drugs, particularly to those acting at the same receptor.

- ▶ Tolerance
- ▶ Tolerance and Desensitization
- ▶ Myasthenia Gravis

Crosslinks

Crosslinks result from the reaction of a bifunctional electrophilic species with DNA bases and imply a covalent link between two adjacent DNA strands which inhibits DNA replication. Primary targets within bases are N7 and O6 in guanine and N3 in cytosine. The initial lesions are removed by the suicide enzyme alkyltransferase, whereas nucleotide excision repair is needed for fully established crosslinks.

- ▶ Antineoplastic Agents

CSFs

- ▶ Colony-stimulating Factors
- ▶ Cytokines
- ▶ Hematopoietic Growth Factors

CTLs

- ▶ Cytotoxic T-cells
- ▶ Immune Defense

Curare

Curare is a generic term for various South American arrow poisons. Curare has been used for centuries by the Indians along the Amazon and Orinoco rivers for immobilizing and paralyzing wild animals used for food. Preparations of curare are derived from *Strychnos* species, which contain quaternary neuromuscular alkaloids like tubocurarine. Tubocurarine is a potent antagonist at the nicotinic acetylcholine receptor.

- ▶ Nicotinic Receptors

Current-voltage Relationship (I-V)

The current-voltage relationship is a plot of the current through a channel versus the voltage of the membrane potential.

- ▶ Voltage-dependent Ca^{2+} Channels
- ▶ Voltage-dependent Na^{+} Channels
- ▶ Voltage-gated K^{+} Channels

Cushing's Syndrome

A hormonal disorder caused by the prolonged exposure to high levels of endogenous or exogenous sources of cortisol.

- ▶ Gluco-mineralocorticoid Receptors
- ▶ Aromatase

Cyclic AMP- and Cyclic GMP-dependent Protein Kinases

Synonyms

cAK or PKAs; cGKs or PKG

Definition

These enzymes are activated by the binding of cAMP or cGMP. When activated, cAKs and cGKs phosphorylate specific serine or threonine residues in target proteins control the activity of these proteins.

- ▶ Adenylyl Cyclases
- ▶ Guanylyl Cyclases
- ▶ Transmembrane Signalling

Cyclic AMP-binding Guanine Nucleotide Exchange Factors

Synonyms

cAMP-GEFs or EPacs

Definition

In the cAMP-bound conformation, cAMP-GEFs specifically bind to Ras-like small GTPases and activate these proteins by profoundly accelerating the exchange of GDP for GTP.

- ▶ Cyclic Nucleotide-regulated Cation Channels
- ▶ Small GTPases
- ▶ Epac

Cyclic-AMP Response Element Binding Protein

- ▶ CREB
- ▶ Antidepressant Drugs

Cyclic Nucleotide-binding Domain

C

Cyclic GMP-dependent Protein Kinase

►Cyclic AMP- and Cyclic GMP-dependent Protein Kinases

Cyclic GMP-regulated Phosphodiesterases

Phosphodiesterases represent a multi-gene family of enzymes that hydrolyze the second messengers cGMP and cAMP. The hydrolytic activity of several sub-families of these enzymes is regulated in an allosteric manner by the binding of cGMP. Notably, the cyclic nucleotide-binding site, present in cGMP-regulated phosphodiesterases is not homologous to that found in most other cyclic nucleotide-binding proteins.

►Cyclic Nucleotide-regulated Cation Channels
►Phosphodiesterases
►Cyclic Guanosine Monophosphate
►Cyclic GMP-dependent Protein Kinase
►Cyclic AMP- and Cyclic GMP-dependent Protein Kinase

Cyclic Guanosine Monophosphate (Cyclic GMP; cGMP)

Synthesized by soluble guanylyl cyclase and particulate guanylyl cyclase from guanosine triphosphate (GTP). Nitric oxide activates soluble guanylyl cyclase to enhance cyclic GMP production that contributes to various NO actions. Cyclic GMP is hydrolyzed by phosphodiesterases. Cyclic GMP binds to and activates ►cGMP-dependent protein kinase, ►phosphodiesterases, and ►Cyclic Nucleotide-regulated Cation Channels.

►Nitric Oxide
►Guanylyl Cyclases
►Phosphodiesterases
►Cyclic Nucleotide-regulated Cation Channels

Synonyms

CNBD

Definition

In cyclic nucleotide-regulated channels, this domain serves as a high-affinity binding site for 3'-5' cyclic monophosphates. The CNBD of channels has a significant sequence similarity to the CNBD of most other classes of eukaryotic cyclic nucleotide receptors and to the CNBD of the prokaryotic catabolite activator protein (CAP). The primary sequence of CNBDs consists of approximately 120 amino acid residues forming three α -helices (αA – αC) and eight β -strands ($\beta 1$ – $\beta 8$).

►Cyclic Nucleotide-regulated Cation Channels
►Cyclic Guanosine Monophosphate

Cyclic Nucleotide Phosphodiesterases

►Phosphodiesterases

Cyclic Nucleotide-regulated Cation Channels

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Synonyms

CNG channels and HCN channels

Definition

Cyclic nucleotide-regulated cation channels are ion channels, whose activation is regulated by the direct binding of cyclic AMP or cyclic GMP to the channel protein. Two families of channels regulated by cyclic nucleotides have been identified, the cyclic nucleotide-gated (CNG) channels [1, 2] and the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels [3–5]. CNG channels require the obligatory binding of a cyclic nucleotide in order to be activated. In contrast, HCN channels are activated by membrane

hyperpolarization. Cyclic nucleotides enhance HCN channel activity by affecting the voltage-dependence of channel activation.

Basic Characteristics

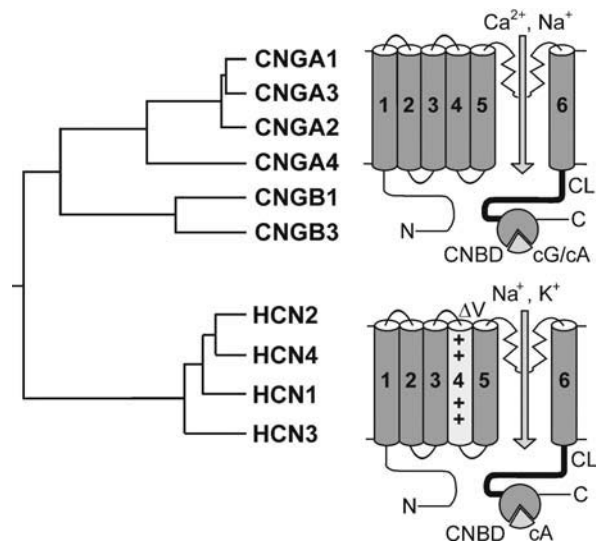
Cyclic nucleotides exert their cellular effects by binding to four major classes of cellular receptors: ►cAMP- and cGMP-dependent protein kinases, cGMP-regulated phosphodiesterases, cAMP-binding guanine nucleotide exchange factors (cAMP-GEFs) and cyclic nucleotide-regulated cation (CNG and HCN) channels. Cyclic nucleotide-regulated cation channels are unique among these receptors because their activation is coupled to the influx of extracellular cations into the cytoplasm and to the depolarization of the plasma membrane. CNG channels pass monovalent cations, such as Na^+ and K^+ , but do not discriminate between them. Calcium is also permeable but at the same time acts as a voltage-dependent blocker of monovalent cation permeability. By providing an entry pathway for Ca^{2+} , CNG channels control a variety of cellular processes that are triggered by this cation. HCN channels conduct Na^+ and K^+ with permeability ratios of about 1:4 and are blocked by millimolar concentrations of Cs^+ . Despite this preference for K^+ conductance, HCN channels carry an inward Na^+ current under physiological conditions. HCN channels can also conduct Ca^{2+} , but not as well as CNG channels. At 2.5 mM external Ca^{2+} , the fractional Ca^{2+} current of HCN2 and HCN4 is about 0.5%, whereas for native CNG channels, it is in the range between 10% and 80%.

CNG and HCN channels belong to the superfamily of ►voltage-gated cation channels. The proposed structure of the channels is shown in Fig. 1. The transmembrane channel core consists of six α -helical segments (S1–S6) and an ion-conducting pore loop between the S5 and S6. The amino- and carboxy-termini are localized in the cytosol. CNG and HCN channels contain a positively charged S4 helix carrying 3–9 regularly spaced arginine or lysine residues at every third position. In HCN channels, as in most other members of the channel superfamily, the S4 helix functions as “voltage-sensor,” conferring voltage-dependent ►gating. In CNG channels, which are only slightly voltage-dependent, the specific role of S4 is not known. In the carboxy-terminus, CNG and HCN channels contain a cyclic nucleotide-binding domain (CNBD) that is homologous to CNBDs of cAKs, cGKs, and cAMP-GEFs. In CNG channels, the binding of cGMP or cAMP to the CNBD initiates a sequence of allosteric transitions that lead to the opening of the ion-conducting pore. In HCN channels, the binding of cyclic nucleotides is not required for activation. However, cyclic nucleotides shift the voltage-dependence of channel activation to a more positive membrane potential and

thereby facilitate voltage-dependent channel activation. CNG and HCN channels are tetramers. In native tissue, HCN channel subunits can assemble to form either homo- or heteromeric complexes. By contrast, all known native CNG channels are heterotetramers.

CNG Channels

CNG channels are expressed in retinal photoreceptors and olfactory neurons, and play a key role in visual and olfactory signal transduction. In addition, CNG channels are found at low density in some other cell types and tissues such as brain, testis, and kidney. While the function of CNG channels in sensory neurons has been unequivocally demonstrated, the role of these channels in other cell types, where expression has been observed, remains to be established. Based on their phylogenetic relationship, the six CNG channels



Cyclic Nucleotide-regulated Cation Channels.

Figure 1 Phylogenetic tree and structural model of cyclic nucleotide-regulated cation channels. The CNG channel family comprises six members, which are classified into A subunits (CNGA1–4) and B subunits (CNGB1 and CNGB3). A “CNGB2” subunit does not exist. The HCN channel family comprises four members (HCN1–4). CNG and HCN channels share the same transmembrane topology, consisting of six transmembrane segments (1–6), a pore loop, and a cyclic nucleotide-binding domain (CNBD). CNG channels conduct Ca^{2+} and Na^+ , whereas HCN channels mainly conduct Na^+ and K^+ . CNG channel is activated *in vivo* by binding of either cAMP (cA) or cGMP (cG), depending on the channel type. HCN channels activate on membrane hyperpolarization (ΔV), and are enhanced by binding of cAMP. The positively charged amino acid residues in the S4 segment of HCN channels are indicated by + symbols. CL, C-linker involved in activation gating of CNG and HCN channels.

identified in mammals are divided into two subfamilies, the A-subunits (CNGA1–4) and the B-subunits (CNGB1 and CNGB3). When expressed in **▶heterologous expression systems**, A-subunits, with the exception of CNGA4, form functional homomeric channels. In contrast, B-subunits do not give rise to functional channels when expressed alone. However, together with CNGA1–3 they confer novel properties (e.g., single channel flickering, increased cAMP sensitivity) that are characteristic of native CNG channels. In native tissues, CNG channels are heterotetramers with different heteromers, displaying distinct nucleotide sensitivity, ion selectivity, and modulation by Ca^{2+} . Recent genetic studies in mice indicate that B-subunits play a key role in principal channel formation and channel targeting in native sensory neurons. For example, mice lacking the CNGB1 subunit fail to express substantial amounts of CNG channels in rod outer segments and olfactory cilia, respectively. The physiological role and subunit composition is known for three native channels: the rod and cone photoreceptor channels and the olfactory channel. The CNG channel of rod outer segment consists of the CNGA1 subunit and the CNGB1a subunit (3:1 stoichiometry). The cone photoreceptor channel consists of the CNGA3 and the CNGB3 subunit (2:2 stoichiometry). CNG channels control the membrane potential and the calcium concentration of photoreceptors. In the dark, the channels are maintained in the open state by a high concentration of cGMP. The resulting influx of Na^+ and Ca^{2+} (“dark current”) depolarizes the photoreceptor and promotes synaptic transmission. Light-induced hydrolysis of cGMP leads to the closure of CNG channels. As a result, the photoreceptor hyperpolarizes and shuts off synaptic glutamate release. Mutations in human CNG channel genes have been linked to retinal diseases. Mutations in the CNGA1 and CNGB1 subunits have been identified in the genome of patients suffering from **▶retinitis pigmentosa**. The functional loss of either the CNGA3 or the CNGB3 subunit causes total color blindness (achromatopsia) and degeneration of cone photoreceptors.

The olfactory CNG channel consists of three different subunits: CNGA2, CNGA4, and the CNGB1b subunit (2:1:1 stoichiometry). The channel is activated *in vivo* by cAMP, which is synthesized in response to the binding of odorants to their cognate receptors. The olfactory CNG channel mainly conducts Ca^{2+} under physiological ionic conditions. The increase in cellular Ca^{2+} activates a Ca^{2+} -activated Cl^- channel, which further depolarizes the cell membrane. Ca^{2+} is not only a permeating ion of the olfactory CNG channel, but also an important modulator of this channel. By forming a complex with calmodulin, which binds to the CNGB1b and CNGA4 subunit, Ca^{2+} decreases sensitivity of the CNG channel to cAMP. The resulting inhibition of

channel activity is the principal mechanism, underlying fast odorant adaptation.

HCN Channels

A cation current that is slowly activated by membrane hyperpolarization (termed I_h , I_f or I_q) is found in a variety of excitable cells, including neurons, cardiac pacemaker cells, and photoreceptors. The best understood function of I_h is to control heart rate and rhythm by acting as “pacemaker current” in the sinoatrial (SA) node. I_h is activated during membrane hyperpolarization, following the termination of an action potential, and provides an inward Na^+ current that slowly depolarizes the plasma membrane. Sympathetic stimulation of SA node cells raises cAMP levels and increases I_h by a positive shift of the current activation curve, thus accelerating diastolic depolarization and heart rate. Stimulation of muscarinic receptors slows down heart rate by the opposite action. In neurons, I_h fulfills diverse functions, including generation of pacemaker potentials (“neuronal pacemaking”), control of membrane potential, generation of rebound depolarizations during light-induced hyperpolarizations of photoreceptors, dendritic integration, and synaptic transmission.

HCN channels represent the molecular correlate of the I_h current. In mammals, the HCN channel family comprises four members (HCN1–4) that share about 60% sequence identity to one another and about 25% sequence identity to CNG channels. The highest degree of sequence homology between HCN and CNG channels is found in the CNBD. The crystal structure of this domain has been determined for HCN2 and a bacterial CNG channel. When expressed in heterologous systems, all four HCN channels generate currents displaying, the typical features of native I_h : (i) activation by membrane hyperpolarization, (ii) permeation of Na^+ and K^+ with a permeability ratio $P_{\text{Na}}/P_{\text{K}}$ of about 0.2, (iii) modulation of voltage-dependence of channel activation by direct binding of cAMP, and (iv) channel blockade by extracellular Cs^+ .

HCN1–4 mainly differ from one another with regard to their speed of activation and the extent to which they are modulated by cAMP. HCN1 is the fastest channel, followed by HCN2, HCN3, and HCN4. Unlike HCN2 and HCN4, whose activation curves are shifted by about +15 mV by cAMP, HCN1, and HCN3 are, if at all, only weakly affected by cAMP.

Site-directed mutagenesis experiments have provided insight into the complex mechanism, underlying dual HCN channel activation by voltage and cAMP. Like in other voltage-gated cation channels, activation of HCN channels is initiated by the movement of the positively charged S4 helix in the electric field. The resulting conformational change in the channel protein is allosterically coupled by other channel domains to the opening of the ion-conducting pore. Major determinants affecting

channel activation are the intracellular S4–S5 loop, the S1 segment, and the extracellular S1–S2 loop. The CNBD fulfills the role of an auto-inhibitory channel domain. In the absence of cAMP, the cytoplasmic carboxy-terminus inhibits HCN channel gating by interacting with the channel core and, thereby, shifting the activation curve to more hyperpolarizing voltages. The binding of cAMP to the CNBD relieves this inhibition. Differences in the magnitude of the response to cAMP among the four HCN channel isoforms are largely due to differences in the extent to which the CNBD inhibits basal **gating**. It remains to be determined if the inhibitory effect of the CNBD is conferred by a direct physical interaction with the channel core domain or by some indirect pathway. There is evidence that the so-called C-linker, a peptide of about 80 amino acids that connects the last transmembrane helix (S6) to the CNBD plays an important role in this process. The C-linker was also shown to play a key role in the gating of CNG channels, suggesting that the functional role of this domain has been conserved during channel evolution.

HCN channels are found in neurons and heart cells. In mouse and rat brain, all four HCN isoforms have been detected. The expression levels and the regional distribution of the HCN channel mRNAs vary profoundly between the respective channel types. HCN2 is the most abundant neuronal channel and is found almost ubiquitously in the brain. In contrast, HCN1, HCN3, and HCN4 are enriched in specific regions of the brain such as thalamus (HCN4) hippocampus (HCN1), or olfactory bulb and hypothalamus (HCN3). HCN channels have also been detected in the retina and some peripheral neurons such as dorsal root ganglion neurons. In SA node cells, HCN4 represents the predominantly expressed HCN channel isoform. In addition, minor amounts of HCN2 and HCN1 are also present in these cells. Insights into the (patho)physiological relevance of HCN channels have been gained from the analysis of mouse lines lacking individual HCN channel isoforms. Disruption of HCN1 impairs motor learning but enhances spatial learning and memory. Deletion of HCN2 results in absence epilepsy, ataxia, and sinus node dysfunction. The mice lacking HCN4 die in utero because of the failure to generate mature sinoatrial pacemaker cells. The key role of HCN4 in controlling heart rhythmicity is corroborated by genetic data from human patients. Mutations in the human HCN4 gene, leading to mutated or truncated channel proteins, have been found to be associated with sinus bradycardia (S672R, 573X) and complex cardiac arrhythmia (D552N).

Drugs Acting on CNG Channels

Several drugs have been reported to block CNG channels. The most widely used among these drugs is *L-cis* diltiazem which blocks CNG channels in a voltage-

dependent manner at micromolar concentrations. The *D-cis* enantiomer of diltiazem, which is an important therapeutic blocker of the *L*-type calcium channel, is much less effective than the *L-cis* enantiomer in blocking CNG channels. High affinity binding of *L-cis* diltiazem is only seen in heteromeric CNG channels, containing the CNGB1 subunit. CNG channels are also moderately sensitive to blockage by some other inhibitors of the *L*-type calcium channel (e.g., nifedipine), the local anaesthetic tetracaine, and calmodulin antagonists. Interestingly, LY83583 [6-(phenylamino)-5,8-quinolinedione] blocks both the soluble guanylyl cyclase and some CNG channels at similar concentrations. H-8 [*N*-2-(methylamino)ethyl-5-isoquinolinesulfonamide], which has been widely used as a nonspecific cyclic nucleotide-dependent protein kinase inhibitor, blocks CNG channels, though at significantly higher concentrations than needed to inhibit protein kinases. The most potent blocking agent for CNG channels is pseudochetoxin. This toxin inhibits homomeric CNGA2 channels with a K_i of 5 nM and the homomeric CNGA1 channel with a K_i of 100 nM. The peptide is several orders of magnitude less effective in blocking the heteromeric channels.

Drugs Acting on HCN Channels

Given the key role of HCN channels in cardiac pacemaking, these channels are promising pharmacological targets for the development of drugs, used in the treatment of cardiac arrhythmias and ischemic heart disease. HCN channels are not expressed in vascular and airway smooth muscle. As a consequence, specific HCN channel blockers are expected to have no side effect on the peripheral resistance. Importantly, unlike the well-established β -adrenoceptor blockers, HCN channel blockers would not impair pulmonary function in patients with asthma or obstructive pulmonary disease. Recently, ivabradine (S16257, Procoralan) was approved as the first therapeutic I_h blocker. Ivabradine blocks cardiac I_h at low micromolar concentrations and is used in the treatment of stable angina pectoris. Other known I_h blockers with blocking mechanisms, related to that of ivabradine are ZD7288 [4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino)pyrimidinium chloride], zatebradine, and cilobradine. These blockers were not introduced into therapy because they either lacked specificity or exerted unacceptable side effects, in particular visual disturbances due to the inhibition of retinal I_h . Interestingly, the well-known α_2 -adrenoceptor agonist clonidine also effectively blocks HCN channels. The block of cardiac I_h (mainly conferred by HCN4) contributes significantly to the bradycardic effect of clonidine. Modulation of I_h may also be a promising approach for the treatment of disease processes in central and peripheral nervous system. For example, I_h is upregulated in dorsal root ganglion neurons in response

to nerve injury, making HCN channels interesting candidates for therapeutic modulation of inflammation and neuropathic pain. Moreover, agents acting on HCN channels may be utilized in the treatment of epilepsies. Finally, HCN1 and HCN2 channels are inhibited by clinically relevant concentrations (≤ 0.5 mM) of the inhalational anesthetics halothane and isoflurane. Similarly, the intravenous anesthetic propofol inhibits and slows the activation of native and expressed HCN channels. Thus, modulation of I_h may contribute to clinical actions of anesthetic agents.

- Cyclic Adenosine Monophosphate
- Table Appendix: Membrane Transport Proteins
- Cyclic Guanosine Monophosphate
- Non-Selective Cation Channels

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Cyclic Nucleotides

Cyclic nucleotides (cAMP and cGMP) are formed enzymatically from the corresponding triphosphates. As ubiquitous second messengers, they mediate many cellular functions which are initiated by first (extracellular) messengers. Their prime targets in eucaryotic cells are protein kinases (►cyclic AMP-dependent protein kinase, ►cyclic GMP-dependent protein kinase), ion channels and enzymes.

- Adenylyl Cyclases
- Guanylyl Cyclases
- Transmembrane Signalling
- Cyclic Adenosine Monophosphate
- Cyclic Guanosine Monophosphate
- Cyclic Nucleotide-gated Channels
- Phosphodiesterases

Cyclin

- Cell Cycle Control

Cyclin-dependent Kinases

- Cell Cycle Control

Cyclooxygenase-2 (Expression in the Kidney)

Cyclooxygenase (COX) activity is responsible for the formation of prostaglandins from their arachidonic acid precursor. Two COX isoforms have been identified, COX-1 and COX-2. While COX-1 is constitutively expressed in most tissues, COX-2 is typically only found after induction by proinflammatory stimuli. However, a constitutively expressed and highly regulated COX-2 is found in the kidney, both in the renal medulla and in the renal cortex. Renal cortical COX-2 is located in the area of the juxtaglomerular apparatus, and prostaglandins formed by COX-2 regulate the expression and secretion of renin in response to a reduction in NaCl concentration at the macula densa.

- Cyclooxygenases
- Diuretics

Cyclooxygenases

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Synonyms

Prostaglandin H₂ synthase (PGHS); EC 1.14.99.1; COX-1; COX-2

Definition

Cyclooxygenase (COX) is the enzyme that catalyses the conversion of arachidonic acid to the highly active group of lipid mediators, the prostaglandins and thromboxane. These end products are involved in a multitude of diverse pathophysiological processes, ranging from the induction of the vascular inflammatory response to tissue damage or infection, fever, pain perception, haemostasis, cytoprotection of the gastric mucosa from the erosive effects of gastric acid, the induction of labour and the regulation of kidney function. Thus, the inhibition of COX can result in alleviation of the symptoms of many diseases, particularly inflammatory joint disease such as ►[rheumatoid arthritis](#). However, this often occurs with the generation of severe side effects, such as gastrototoxicity, due to the inhibition of physiological functions.

In 1971 inhibition of COX was established as the mode of action of the archetypal non-steroid anti-inflammatory drug (NSAID) aspirin, thus resolving an enigma that had puzzled researchers since the synthesis of salicylate in the mid nineteenth century. The realisation that inhibition of COX was the general mode of action of the large number of marketed NSAIDs also explained why the common toxic effects of these drugs, such as gastro- and nephrotoxicity, prolongation of bleeding time and delayed labour, could not be divorced from their beneficial analgesic, antipyretic and anti-inflammatory actions.

Basic Characteristics

COX possesses two catalytic sites. The cyclooxygenase active site converts arachidonic acid substrate, mobilised from membrane phospholipid by a phospholipase, to the prostaglandin endoperoxide, PGG₂. The endoperoxide is then converted by a peroxidase-active site on COX to form prostaglandin H₂ (PGH₂). PGH₂ is then acted upon by various synthases to form the active prostanoids, the most important of which are prostaglandin E₂ (PGE₂), thromboxane A₂ (TXA₂), prostacyclin (PGI₂), prostaglandin D₂ (PGD₂) and prostaglandin F_{2α} (PGF_{2α}). COX is widely distributed in tissues, indeed, prostanoids can be synthesised and released by any mammalian cells except erythrocytes. The stages in the production of the prostanoids are summarised in [Fig. 1](#). The main actions of these mediators are listed in [Table 1](#).

By the late 1980s, it became clear that there were two forms of COX, one that was constitutive, i.e. permanently present in many tissues, and the other that could be induced over time by mitogens or pro-inflammatory agents, this induction being inhibited by

anti-inflammatory steroids such as dexamethasone. In 1991, it was established that there were, in fact, two distinct COX enzymes, encoded by two distinct genes. The constitutive enzyme was designated COX-1 and the inducible enzyme COX-2. In general, COX-1 is responsible for the synthesis of PGs, which serve physiological or so-called “house-keeping” functions, whereas COX-2 synthesises PGs, involved in pathological processes, which are responsible for symptoms such as the swelling and pain of inflammatory conditions.

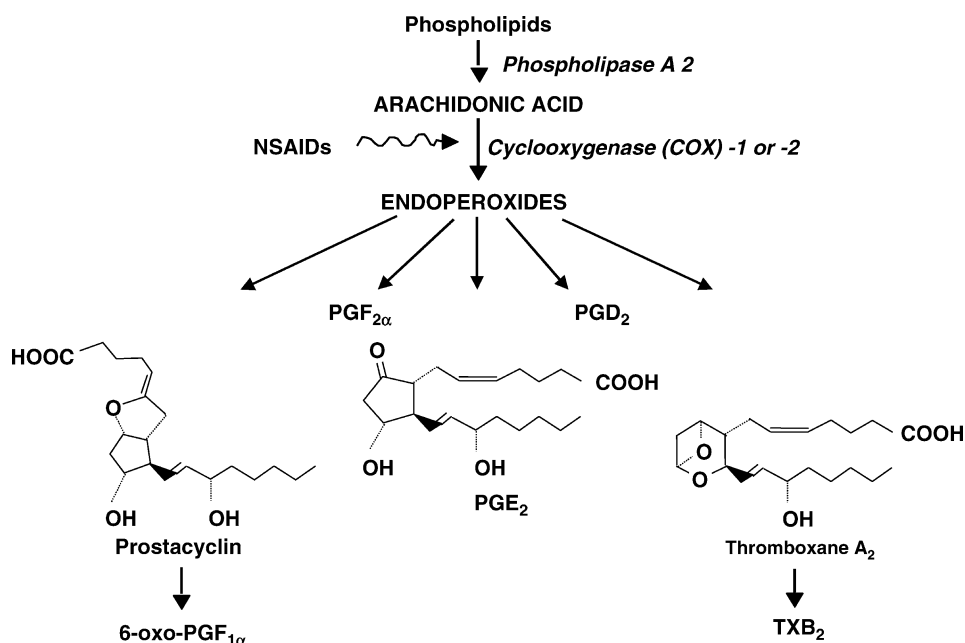
The two isozymes are both homodimers, composed of approximately 600 amino acids and possess approximately 60% homology. The three-dimensional structures of COX-1 and COX-2 are very similar. Each one consists of three independent units: an epidermal growth factor-like domain, a membrane-binding section and an enzymic domain. The catalytic sites and the residues immediately adjacent are identical but for two small but crucial variations that result in an increase in the volume of the COX-2-active site, enabling it to accept inhibitor-molecules larger than those that could be accommodated in the COX-1 molecule.

The identification of the two COX isozymes explained why some NSAIDs had the same efficacy as other drugs, but apparently, were marginally less likely to produce the common side effect of gastrotoxicity, manifested by perforations, ulcers or bleeding from the gastric mucosa. It is of greater significance that the establishment of differences in the volume of the active site of COX-2 also raised the possibility of the synthesis of inhibitors, tailor-made to fit the COX-2 site, without the capacity to inhibit COX-1. Such drugs would prevent the formation of PGs responsible for pain, fever and inflammatory reactions, with minimal or no action on the synthesis of the beneficial or “housekeeping” PGs ([Fig. 2](#)).

Since COX-2 is overexpressed in tumour cells, such as those of colorectal cancer, it was anticipated that selective COX-2 inhibitors may inhibit tumour growth.

Drugs

Aspirin has been remarkably successful in the treatment of the pain and swelling of inflammatory disease and in fact, an estimated 45,000 tons of aspirin are still consumed each year. This success resulted in the syntheses of many other “aspirin-like drugs”, now referred to as NSAIDs. Aspirin, however, continues to have a unique use in the prevention of thrombosis. Since it produces irreversible inhibition of COX-1 by acetylation of serine at position 530 in the active site, a daily low dose of aspirin will cause a cumulative inhibition of COX-1 in platelets, in the portal circulation. A gradual inhibition of platelet aggregation occurs, reducing the possibility of occlusion of coronary or cerebral vessels by platelet thrombi. However, there are no systemic



Cyclooxygenases. Figure 1 Pathways for the formation of prostanoids from arachidonic acid. Arachidonic acid is converted by cyclooxygenase to endoperoxides, which are acted upon by various synthetases to form the prostanoids. Prostacyclin and thromboxane are relatively unstable and break down rapidly to form the inactive metabolites 6-oxo-PGF_{1α} and thromboxane B₂, respectively.

Cyclooxygenases. Table 1 Actions of the common prostanoids

Actions of the common prostanoids	
PGE ₂	Vasodilatation (decrease in blood pressure); inhibits platelet aggregation; decreased gastric secretion; bronchodilatation; hyperalgesia; fever; contraction of intestinal and uterine smooth muscle. (Mediates inflammation, fever, pain, protects gastric mucosa)
PGI ₂	Vasodilatation (decrease in blood pressure); inhibits platelet aggregation; decreased gastric secretion; bronchodilatation; hyperalgesia. (Mediates pain, protects gastric mucosa, antithrombogenic)
PGF _{2α}	Vasodilatation/vasoconstriction; bronchoconstriction; contraction of intestinal and uterine smooth muscle. (Important functions in ovulation and parturition)
PGD ₂	Vasodilatation; inhibits platelet aggregation; decreases fever; relaxes intestinal smooth muscle. (Released mainly in brain and mast cells, induces sleep)
TXA ₂	Increases blood pressure; platelet aggregation; vasoconstriction; bronchoconstriction. (Important in haemostasis)

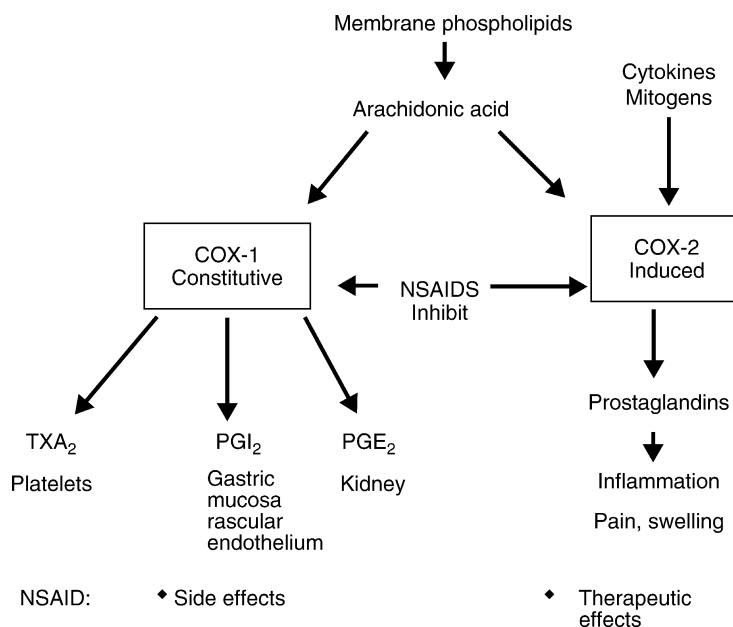
Putative pathophysiological functions placed in parenthesis.

toxic effects since the small dose of aspirin administered is destroyed by hepatic metabolism.

NSAIDs are used as the first-line treatment of rheumatoid arthritis, ▶osteoarthritis, systemic lupus erythematosus and other inflammatory diseases, and are thus amongst the most widely used drugs in the developed world. This widespread use inevitably entailed a considerable associated morbidity, in particular a high incidence of gastric toxicity. In the USA alone, perforations, ulcers and bleeds lead to the hospitalisation of 100,000 patients per year, and about 15% of these die while under intensive care.

NSAIDs are of diverse chemical structures: salicylates (aspirin, sulphasalazine), indole acetic acids (indomethacin, etodolac), heteroaryl acetic acids (diclofenac), arylpropionic acids (ibuprofen, naproxen), anthranilic acids (mefenamic acid) and enolic acids (piroxicam, meloxicam).

The identification of COX-2 as the enzyme, responsible for the synthesis of PGs involved in pathology, led to the examination of the relative activities of the established NSAIDs on COX-1 and COX-2. Various test systems have been used for this purpose and it is now generally accepted that the whole



Cyclooxygenases. Figure 2 Mechanism of the therapeutic and toxic actions of NSAIDs. The induced cyclooxygenase (COX-2) produces the pro-inflammatory prostaglandins responsible for the swelling and pain associated with inflammation. COX-1 produces the prostanooids responsible for protective physiological actions: thromboxane A₂ (TX)A₂ causes platelet aggregation, PGI₂ prevents intravascular aggregation and is protective for the gastric mucosa, and PGE₂ maintains renal blood flow (from Botting, 1999, with permission) [1].

blood assay of Patrignani et al. is the preferred method since it uses the human enzymes in platelets (COX-1) and activated mononuclear cells (COX-2) in the presence of the plasma proteins, thus allowing for the participation of protein binding which is significant for some NSAIDs [2].

Three established NSAIDs were found to inhibit COX-2 in lower concentrations than those required to inhibit the constitutive enzyme. These were nimesulide, etodolac and meloxicam. Epidemiological studies showed that at therapeutic doses these drugs induced less gastrointestinal toxicity than NSAIDs, such as indomethacin, naproxen and ketorolac, which were more potent at inhibiting COX-1 compared with COX-2. The proof that selective inhibitors of COX-2 are efficacious anti-inflammatory agents with minimal or no toxic side effects, stimulated several commercial groups to develop, through knowledge of the differences in the shape of the catalytic sites of the isozymes, highly selective inhibitors of COX-2, some of which are 1000-fold more potent against COX-2 than COX-1.

The first two selective COX-2 inhibitors to be marketed and subjected to in depth clinical trials were celecoxib and rofecoxib. Both compounds are as effective as standard NSAIDs in rheumatoid arthritis, osteoarthritis and for pain following orthopaedic or dental surgery. Gastrointestinal side effects were far fewer than with comparator drugs and in fact were no

more common with the ►coxibs than with placebo treatment.

However, already in an early clinical trial, rofecoxib was found to produce four times the number of ►myocardial infarctions than its comparator drug, naproxen. A subsequent trial of rofecoxib compared to placebo in colorectal cancer prevention demonstrated, after 18 months of study, that a greater number of myocardial infarctions occurred in the rofecoxib group. In 2004 the manufacturers of rofecoxib withdrew the drug from the market. A similar study of celecoxib compared to placebo in cancer prevention, showed that celecoxib also increased the risk of cardiovascular embolisms [3].

The Future of Selective COX-2 Inhibitors

Celecoxib, which has a low selectivity for COX-2 compared to COX-1, is still available, although its more selective successor, valdecoxib has been withdrawn. Etoricoxib, the successor to rofecoxib, is marketed in Europe but not in the USA. In a large multinational clinical trial, etoricoxib caused no more thromboembolic events than diclofenac, but after 18 months the incidence of gastrointestinal ulcers and bleeding was the same for both drugs [4].

Lumiracoxib, which has been approved in the UK but not by the FDA, has a phenyl acetic acid structure resembling diclofenac rather than the other coxibs

which are sulphonamide or sulphone derivatives. It is highly selective for COX-2 and studies show a similar incidence of thromboembolism and lower incidence of gastrointestinal damage than comparator NSAIDs.

COX-2 synthesises PGI₂ (prostacyclin) and the high incidence of myocardial infarctions with selective COX-2 inhibitors has been attributed to inhibition of COX-2 in vascular tissues. Prostacyclin, made by blood vessel walls, inhibits aggregation of platelets and maintains a balance with thromboxane. Thromboxane, which is released by platelets, promotes clotting. Prostacyclin is synthesised mostly by COX-1, but in humans selective COX-2 inhibition reduces its biosynthesis *in vivo*. This reduced synthesis may lead to an overactive thromboxane system and increased risk of thromboembolism.

Current studies indicate that prolonged use of selective COX-2 inhibitors, which would be required to treat cancer, may lead to an increase in myocardial infarctions and ultimately, gastric damage. The indications are that selective COX-2 inhibitors relieve the pain of rheumatoid arthritis, osteoarthritis and acute pain, but they should not be administered continuously for long periods. They should not be given to patients for pain originating in the cardiovascular system, for example, pain following cardiac arterial bypass surgery.

The possibility still exists that selective COX-2 inhibitors may be used to treat cancer if the beneficial effect outweighs the side effects. They may also have a therapeutic role in treating premature labour, since labour is induced partly through the uterotonic effect of PGs synthesised by COX-2. Non-selective NSAIDs such as indomethacin will also delay premature labour but they are contraindicated for this condition since they also cause early closure of the ductus arteriosus through inhibition of COX-1, which synthesises PGs maintaining patency of the ductus [5].

- ▶ Diuretics
- ▶ p450 Mono-Oxygenase System
- ▶ Prostanoids
- ▶ Inflammation
- ▶ Non-steroidal Anti-inflammatory Drugs

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Cyclosporin A

A secondary metabolite produced by *Tolypocladium inflatum*. This fungus was initially isolated in a soil sample collected in Norway. Cyclosporin A is a cyclic undecapeptide. Inside cells, cyclosporine A binds its immunophilin receptor known as cyclophilin. Like the FK506-FKBP12 complex, cyclosporin A-cyclophilin binds and inhibits the protein phosphatase calcineurin.

- ▶ Immunosuppressive Agents
- ▶ TOR Signalling

Cysteine Endopeptidases

- ▶ Cystein Proteinases

Cysteine Proteinases

Synonyms

Cysteine proteases; Cysteine endopeptidases

Definition

Cysteine proteinases are proteinases that utilize the terminal sulfhydryl moiety of the side chain of cysteine to effect peptide bond hydrolysis.

- ▶ Non-viral Peptidases
- ▶ Caspases
- ▶ Calpains
- ▶ SUMoylation

Cysteinyl Leukotriene

Cysteinyl leukotriene is a compound synthesized from arachidonic acid in inflammatory cells that contains an amino-acid side chain.

- ▶ Leukotrienes
- ▶ Cyclooxygenases

Cystic Fibrosis

Cystic fibrosis is a common autosomal recessive disorder caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The CFTR protein is a cAMP-regulated Cl^- channel belonging to the ABC transporter family. The main symptoms of cystic fibrosis are a progressive obstructive lung disease and pancreatic insufficiency. Patients suffer from the increased production of viscous mucus which leads to impaired function and severe infections of the lung. About 70% of the patients carry the $\Delta F508$ mutation which leads to an intracellularly-retained CFTR protein. Cystic fibrosis is one of the most common hereditary diseases of children that reduce life expectancy.

- ▶ Cl^- Channels and Cl^-/H^+ Exchangers
- ▶ Epithelial Na^+ Channel
- ▶ Protein Trafficking and Quality Control

Cytochalasins

Cytochalasins B and D are used as tools to study F-actin. Cytochalasins bind to the barbed end of F-actin and block the addition as well as dissociation of G-actin at that end. When applied to cultured cells micromolar concentrations of cytochalasins remove stress fibres and other F-actin structures.

- ▶ Cytoskeleton

Cytochrome P450

- ▶ P450 Mono-oxygenase System

Cytochrome P450 2C9

Cytochrome P450 2C9 is a mixed-function oxidase localized in the endoplasmic reticulum which is responsible for the biotransformation of several nonsteroidal anti-inflammatory drugs, S-warfarin, several sulfonylurea antidiabetics and other drugs.

- ▶ Pharmacogenetics
- ▶ P450 Mono-oxygenase System

Cytochrome P450 2C19

Cytochrome P450 2C19, also termed S-mephenytoin hydroxylase, is a mixed-function oxidase localized in the endoplasmic reticulum which is responsible for the biotransformation of S-mephenytoin, some barbiturates, almost all proton pump inhibitors such as omeprazole, diazepam and others.

- ▶ Pharmacogenetics
- ▶ P450 Mono-oxygenase System

Cytochrome P450 2D6

Cytochrome P450 2D6, also termed debrisoquine-sparteine hydroxylase, is a mixed-function oxidase localized in the endoplasmic reticulum which is responsible for the biotransformation of several tricyclic antidepressants, antipsychotics, beta-blockers, opioids, and many other drugs.

- ▶ Pharmacogenetics
- ▶ P450 Mono-oxygenase System

Cytochrome P450 Induction

Cytochrome P450 induction is the process whereby cellular and tissue levels of one or more cytochrome P450 enzymes are increased in response to treatment of cells, or a whole organism, with certain drugs or environmental chemicals referred to as cytochrome P450 inducers. Cytochrome P450 induction leads to an increase in the cell's capacity for P450-catalyzed oxidative metabolism of many xenochemicals, as well as endogenous steroidal and fatty acid cytochrome P450 substrates.

- ▶ Pharmacogenetics
- ▶ P450 Mono-oxygenase System

Cytochrome P450 Isozymes

- ▶ P450 Mono-oxygenase System

Cytochrome P450 Mono-oxygenases

- ▶ P450 Mono-oxygenase System

Cytokeratin

Cytokeratins are members of the intermediate filament class of cytoskeletal proteins. Cytokeratins are a large protein family comprising two subfamilies of polypeptides, i.e. acidic (type I) and basic (type II) ones. Cytokeratin form tetramers, consisting of two type I and two type II polypeptides arranged in pairs of laterally aligned coiled coils. The distribution of the different type I and II cytokeratins in normal epithelia and in carcinomas is differentiation-related and can be used for cell typing and identification.

- ▶ Cadherins/Catenins
- ▶ Cytoskeleton

Cytokine Receptors

Cytokine receptors are a group of structurally related receptors, which couple to the JAK-STAT pathway. Cytokine receptors function as homodimers or hetero-oligomers. They are divided into two main subclasses, class I, which contains receptors for a variety of hematopoietic growth factors and interleukins and class II, which contains receptors for interferons and interleukins 10, 20/24 and 22.

- ▶ Cytokines
- ▶ JAK-STAT Pathway
- ▶ Table appendix: Receptor Proteins

Cytokines

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Synonyms

No true synonyms. Related terms/subgroups: Lymphokines; Monokines; Interleukins; Chemokines; Interferons; Growth factors ([Table 1](#)).

Definition

Cytokines are a large and heterogenous group of small polypeptides, most of them with a Mr between 8 and 30 kDa. They are mostly soluble extracellular mediators (some exist in membrane-associated form) and function through high-affinity cell surface receptors. Released by cells of hematopoietic origin (reflected in the terms “lymphokines” and “monokines”) as well as by a wide variety of nonhematopoietic cells, cytokines are critical for the normal functioning of the immune defense, which they coordinate by communicating between participating cells.

“My private metaphor was that of a zoo of factors in a jungle of interactions surrounded by deep morasses of acronyms and bleak deserts of synonyms” [1].

Basic Characteristics

Historically, many cytokines were discovered by means of their immunoregulatory properties, hence, the term interleukins. However, cytokines regulate not only functions of the specific immune system, but also many

Cytokines. Table 1 Structural/functional groups of cytokines (examples)

Interferons	IFN- α , β , γ
Hematopoietic cytokines/colony-stimulating factors	IL-3, IL-5, IL-6, IL-7, erythropoietin, GM-CSF, G-CSF, M-CSF, thrombopoietin
IL-1 family	IL-1 α , IL-1 β , IL-18, IL-1RA
TNF family	TNF- α , TNF- β , CD40 ligand, CD95 ligand, TRAIL
Chemokines	IL-8, MIP-1- α , RANTES
Immunoregulatory cytokines	IL-2, IL-4, IL-10, IL-12

aspects of infection and inflammation. At the cellular level they control proliferation, differentiation, functional activity, and apoptosis. As a whole, they are synthesized by a broad variety of different cell types. This also holds true for many cytokines individually (e.g., IL-6, IL-8, IFN- β , for further information on individual cytokines see [1]), whereas synthesis of others is restricted to one or few cell types (e.g., IL-2, IL-4, IFN- γ). In general, their biosynthesis is rapidly and highly inducible by changes in the cell microenvironment. Cytokines often have a short half-life and production is restricted to potentially pathological conditions. Uncontrolled synthesis results in chronic inflammatory or other diseases. All cytokines act locally in an autocrine or paracrine manner, but some may also act systemically. Examples for the latter are the induction of fever by IL-1 and $\blacktriangleright$ **TNF**, and the induction of acute phase proteins in the liver by IL-6. Cytokines can therefore also be regarded as hormones. Cytokines are pleiotropic, that is, they control multiple biological responses. There is considerable functional overlap between certain cytokines (e.g., $\blacktriangleright$ **TNF** and IL-1). In general, activation of a cell by a cytokine results in reprogramming of the cell's gene expression profile.

Mechanism of Action

Most cytokines – in particular the ones that are in clinical use – bind to high-affinity receptors on the plasma membrane. These receptors fall into different classes, those with enzymatic tyrosine kinase activity (e.g., the receptors of certain hematopoietic growth factors (M-CSF, SCF), G-protein coupled receptors (chemokine receptors), and several groups that bind to other intracellular adaptor proteins (IL-1-, TNF-, interferon- and hematopoietic cytokine receptor families). Ligand-dependent receptor clustering activates several intracellular signaling pathways. These pathways consist of consecutive interactions between protein components that in part involve enzymatic activities. The activity of many proteins in a given cytokine-activated pathway is regulated by reversible phosphorylation. The $\blacktriangleright$ **MAP kinase** cascades and the $\blacktriangleright$ **JAK/STAT** pathways are intensively studied exam-

ples. Certain receptors (of the TNF-R family) can activate caspases; a family of proteases that executes programmed cell death. Other protein synthesis independent effects of cytokines include cytoskeletal changes and modulation of cell surface receptors and adhesion molecules.

Most cytokine-activated intracellular pathways ultimately turn on expression of genes whose products change the biological function of the same or a neighboring cell. These include cytokines (hence the term “cytokine network”), cell surface receptors and adhesion molecules, enzymes involved in degradative processes (e.g., collagenases) and in formation of small molecular weight mediators (e.g., cyclooxygenase II, inducible NO-synthase).

Cytokines and Therapy

Knowledge on cytokines has been exploited to improve the therapy of many diseases, basically in two ways:

1. Application of cytokines as drugs. Several examples will be given below. In general, treatment with cytokines turned out to be problematic due to their pleiotropic nature of function. Thus tumor necrosis factor, apart from its antitumoral effect, has severe systemic side effects as manifestations of its activity, which largely precludes its use as anticancer drug. Similar toxicity has been observed for several other inflammatory cytokines. Recombinant cytokines can be used to boost immune reactions during infection and cancer (interleukin-2, interferons) or to substitute cytokine deficiencies (hematopoietic growth factors, erythropoietin).
2. Interference with cytokine action. Inhibition of cytokine synthesis has turned out to be a major component of the activity of anti-inflammatory drugs, glucocorticoids being a prominent example. Because for many cytokines the molecular mechanisms of their actions have been worked out in detail, rational strategies for interfering with their biological activities have been or are being developed. The action of cytokines can be suppressed pharmacologically by different means: (i) Inhibition of their synthesis by drugs (cyclosporin-IL-2 synthesis); (ii) Prevention of their interaction with cell

surface receptors by soluble receptors (TNF-RI extracellular domain), anticytokine, or anticytokine receptor antibodies (anti-TNF, anti-IL-2R antibodies), or natural antagonists (IL-1R antagonist); (iii) Blockade of specific events in the intracellular signaling pathways (protein kinase inhibitors). Clinically used cytokines or cytokine antagonists are proteins. Repeated application, especially for those of nonhuman origin, bears the risk of recognition by the immune system and antibody production against them with subsequent loss of efficacy. Therefore, the proteins are often genetically engineered, that is, humanized (see Humanized Monoclonal Antibodies) to be as homologous as possible to human proteins.

Clinical Use (Including Side Effects)

Examples of Recombinant Cytokines Currently in Use

Interferons

Interferon alfacon-1 (Inferax®), interferon alfa-2b (IntronA®), and interferon alfa-2a (Roferon®-A) are applied in the treatment of chronic hepatitis B and C and some malignancies, especially hairy cell leukemia. IFN- α proteins induce the expression of antiviral, antiproliferative and immunomodulatory genes.

Interferon beta-1a (AVONEX®, Rebif®), interferon beta-1b (Betaferon®), and interferon beta (Fiblaferon®) are applied in multiple sclerosis to reduce both frequency and severity of disease incidents and for the treatment of severe viral infections. In multiple sclerosis, IFN- β proteins modulate the destruction of myelin in the cause of the autoimmune reaction.

Interferon gamma-1b (Imukin®) is used to reduce the incidence of infections in the treatment of chronic granulomatosis.

Common side effects of interferons are flu-like symptoms, fever, myelosuppression, and skin-reactions.

Human Recombinant Colony-Stimulating Factors

Molgramostim (rhu GM-CSF, Leucomax®), Filgrastim (r-metHuG-CSF, Neupogen®), and Lenograstim (rHuG-CSF, Granocyte®) promote the differentiation of pluripotent bone marrow stem cells to leukocytes. GM-CSF induces proliferation in cells of the macrophage, neutrophil, and eosinophil lineages, while G-CSF acts primarily on neutrophil precursors. They are effective in treatment of congenital and acquired neutropenias during chemotherapy of cancer or bone marrow transplantation. G-CSF is also applied to donors to increase peripheral blood progenitor cells for allogeneic bone marrow transplantation. Side effects include bone pain, fever, and myalgia.

Interleukin 2 (Aldesleukin, Proleukin®) is a major growth factor and activator of cytotoxic and other T-lymphocytes. It is applied in the therapy of metastasizing renal carcinoma and melanoma. Side effects include

hypotension, arrhythmias, edema, pruritus, erythema, central nervous symptoms, fever, and many others.

Erythropoietin (Eprex®) is physiologically produced in the kidney and regulates proliferation of committed progenitors of red blood cells. It is used to substitute erythropoietin in severe anemias due to end stage renal disease or treatment of cancer with cytostatic agents. Side effects include hypertension and increased risk of thrombosis.

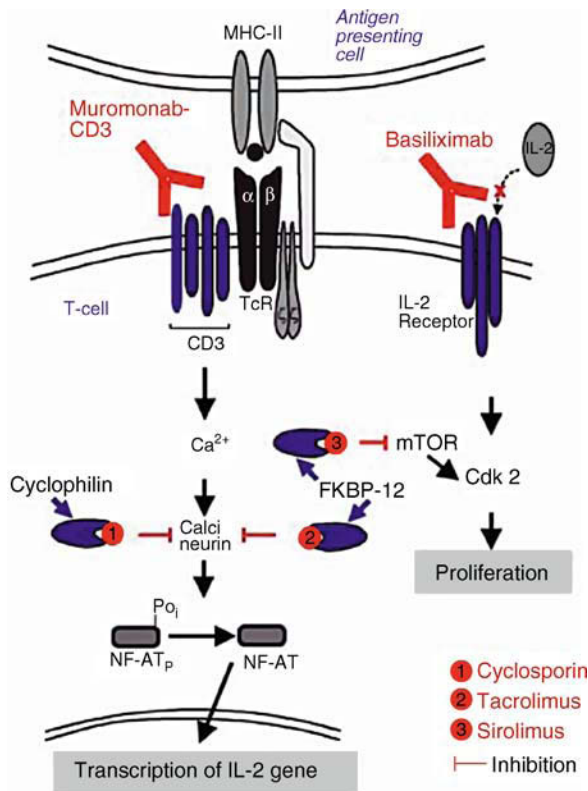
Inhibitors of Cytokine Action

Inhibition of hematopoietic growth factors: Imatinib (Glivec®) is applied to treat chronic myeloid leukemia in Philadelphia-chromosome positive patients. In these patients, translocation of parts of chromosomes 9 and 22 results in the expression of a fusion protein with increased tyrosine kinase activity, called Bcr-Abl. Imatinib is a small Mw inhibitor selective for the tyrosine kinase activity of Bcr-Abl. Thereby, it inhibits the Bcr-Abl induced cell cycle progression and the uncontrolled proliferation of tumor cells.

Inhibition of immunomodulatory cytokines (Fig. 1): Anti-T-cell receptor antibodies: Muromonab (OKT3, Orthoclone®) binds to the CD3 complex of the T-cell receptor and induces depletion of T-lymphocytes. It is applied to prevent acute rejection of kidney, liver, and heart allografts. Rapid side effects (within 30–60 min) include a cytokine release syndrome with fever, flu-like symptoms, and shock. Late side effects include an increased risk of viral and bacterial infections and an increased incidence of lymphoproliferative diseases due to immunosuppression.

Humanized recombinant anti-IL-2 receptor antibodies (Basiliximab, Simulect®, and Daclizumab Zenapax®). These antibodies bind with high affinity to the IL-2 receptor on T-lymphocytes and prevent activation and clonal expansion of anti-allograft T-lymphocytes by endogenous IL-2. They are used to prevent kidney allograft rejection. The main side effect is immunosuppression.

Intracellular inhibition of interleukin-2 production and activity: The immunosuppressants Cyclosporin (Sandimmune®), Tacrolimus (Prograf®), and Sirolimus (Rapamune®) are used for the prophylaxis of allograft rejection. Cyclosporin binds to the intracellular protein cyclophilin, and Sirolimus binds to the intracellular protein FKBP12. The resulting complexes inhibit a serine-threonine phosphatase, calcineurin, which is required for T-lymphocyte activation and for IL-2 gene expression. Sirolimus also binds to FKBP12. However, the complex inhibits a different enzyme, the protein kinase mTOR (mammalian target of rapamycin); thereby, it inhibits IL-2-dependent progression of activated T-lymphocytes through the cell cycle. All three drugs suppress clonal expansion of antigen-activated T-lymphocytes. Cyclosporin and Tacrolimus



Cytokines. Figure 1 Inhibition of cytokine synthesis during activation of the specific immune system. The monoclonal antibodies Muromonab and Basiliximab are specific for the CD3 complex of the T-cell receptor, and for the IL-2 receptor on lymphocytes, respectively. Cyclosporin and Tacrolimus inhibit activation of cytoplasmic NF-AT, a transcription factor essential for activation of the IL-2 gene (►NFAT Family of Transcription Factors). Sirolimus interferes with mTOR signaling and inhibits IL-2 dependent proliferation. Red: pharmaka, blue: target proteins.

are nephrotoxic, and Sirolimus causes hyperlipidemia. For further details see ►Immunosuppressive Agents.

Inhibition of inflammatory cytokines (Fig. 2): Humanized monoclonal anti-TNF antibodies (Infliximab (Remicade®), Adalimumab (Humira®)) bind with high selectivity to human TNF- α and neutralize its activity. Thereby, infliximab decreases the effects of enhanced TNF levels during inflammatory disease such as production of proteases, chemokines, adhesion molecules, cyclooxygenase products (prostaglandins), and proinflammatory molecules such as interleukin-1 and -6. The antibodies may also recognize membrane-bound TNF- α on lymphocytes and other immune cells. These cells may subsequently become apoptotic or are eliminated via Fc-receptor-mediated phagocytosis.

The antibodies are used to treat Crohn's disease, Psoriasis (including Psoriasis arthritis), Spondylitis ankylosans, and rheumatoid arthritis. Side effects

include immunosuppression and increased risk of infections.

Recombinant soluble TNF-RI-IgG1 fusion protein Etanercept, Enbrel® is a chimeric molecule consisting of the extracellular domain of the TNF receptor I (TNF-RI) and the Fc portion of human IgG1. Two Fc domains are bound to each other via disulfide bonds, thereby yielding dimers with two binding sites for the TNF trimer. Etanercept binds with high affinity to extracellular TNF and reduces TNF activity. Etanercept is not effective in Crohn's disease, possibly because it does not lead to destruction of membrane TNF- α expressing cells. Indications and side effects are similar to those of Infliximab and Adalimumab.

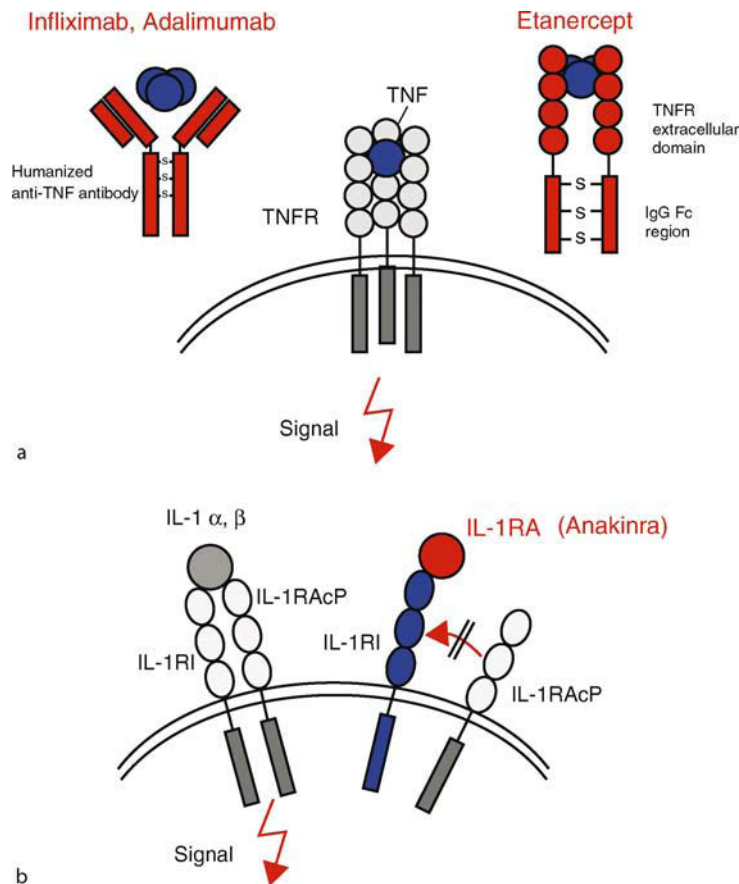
Recombinant human IL-1 receptor antagonist (Anakinra, Kineret®) blocks the biological activity of interleukin-1 by competitively inhibiting IL-1 binding to the interleukin-1 type I receptor (IL-1RI), which is expressed in a wide variety of tissues and organs. Thereby it reduces the pro-inflammatory activities of IL-1 including cartilage destruction and bone resorption. Side effects include an increased risk of infections and neutropenia.

Outlook: The anti TNF and anti IL-1 therapies are being improved by modifications such as addition of polyethylene glycol (PEG) to optimize pharmacokinetic parameters or by the development of inhibitors that can be administered orally. The concept of genetically engineered antibodies or cytokine traps is currently being expanded to include many more cytokines (e.g., IL-6 and IL-4). Many different protein kinase inhibitors that target central effector molecules in the intracellular signaling pathways downstream of cytokine receptors are in different phases of clinical testing. It can be expected that some of them – like the inhibitors of IL-2 signaling described above – will be approved soon to treat human inflammatory disease.

- Chemokine Receptors
- Immune Defense
- JAK-STAT Pathway
- PIAS proteins
- Map Kinase Cascades
- Toll-like Receptors
- Growth Factors
- Tumor Necrosis Factor (TNF)
- Immunosuppressive Agents

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Cytokines. Figure 2 Cytokines. Strategies directed against TNF or IL-1 in anti-inflammatory therapy.

(a) TNFRI-IgGFc fusion proteins or monoclonal anti-TNF antibodies capture TNF and prevent its binding to cell surface receptors. (b) The IL-1 receptor antagonist (IL-1Ra) is a natural ligand that occupies the IL-1 receptor type I (IL-1RI). Thereby it prevents the IL-1-induced formation of a signal-inducing heterodimeric complex with the IL-1 receptor accessory protein (IL-1RAcP).

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Cytokinesis

At the end of mitosis after re-establishment of the cell nuclei the cytoplasm is divided resulting in two independent daughter cells.

► Cell Cycle Control

Cytomegalovirus CMV

Cytomegalovirus (CMV) is a herpesvirus, which causes an inapparent infection in immunocompetent persons. Worldwide, approximately 40% of people are infected with CMV. In immunocompromised patients, transplant recipients and neonates, CMV can cause serious and potentially lethal disease manifestations like pneumonia, retinitis and blindness, hepatitis, infections of the digestive tract, deafness or mental retardation.

► Antiviral Drugs

Cytoplasmic Tyrosine Kinases

► Tyrosine Kinases

Cytoskeleton

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Synonyms

F-actin; Actin filaments; Microfilaments

Definition

► **Microtubules**, ► **F-actin** (microfilaments) and ► **intermediate filaments** form the cytoskeleton. These polymeric structures are highly dynamic and respond to extracellular signals. Thus, they mediate the adaptation of the cellular architecture to functional changes. They are essential for cell attachment, motility and proliferation, and organize the intracellular transport and compartments. In addition, cytoskeletal elements anchor receptors and ion channels in the cell membrane and organize the subsequent signal transduction pathways. Several drugs are known that specifically affect microtubules and microfilaments. In contrast, the specific pharmacological manipulation of intermediate filaments is presently not possible. Therefore, the present chapter will not deal with intermediate filaments.

Basic Characteristics

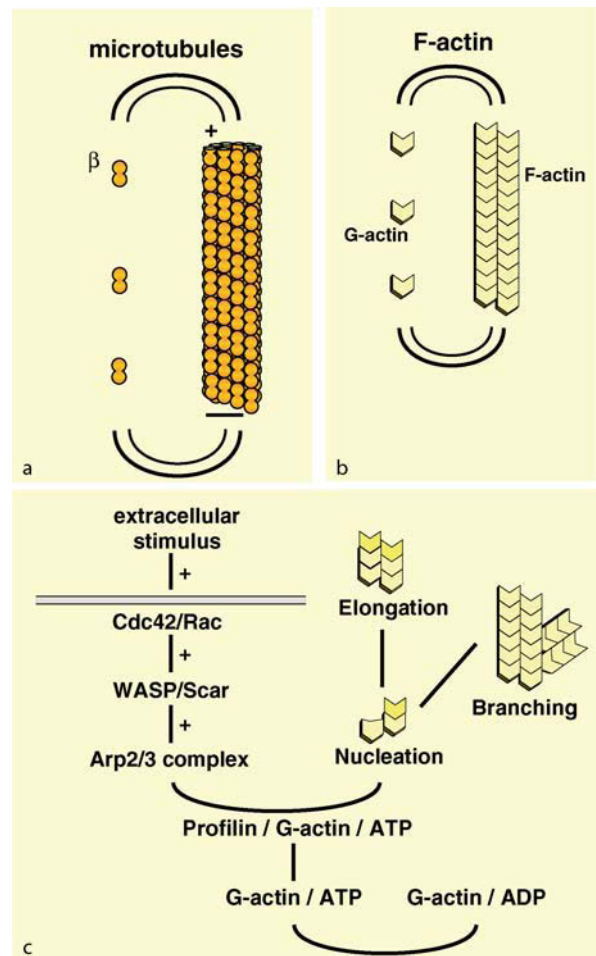
Organization of Microtubules

The globular polypeptides α - and β -**tubulin** have molecular weights of about 50 kDa. In mammals 6 known α -tubulin and 7 known β -tubulin genes are expressed in a cell-type specific manner. Whereas α - as well as β -tubulins bind GTP, only the GTP bound by β -tubulin is exchangeable. α - and β -tubulins associate to heterodimers that polymerize head-to-tail to form protofilaments, 13 of which make up a hollow tube of 25 nm diameter, a microtubule (MT) (**Figure 1a**). These polarized structures have GTP-binding β -tubulins at the plus ends and α -tubulins at the minus ends. β -tubulins within the protofilament are in the GDP-binding state. Heterodimers can be added or released during processes called “dynamic instability” and “treadmill-

ing.” Dynamic instability of MTs is characterized by abrupt stochastic transitions among phases of growing, shortening and pause. In most cells, the plus end grows and shrinks, while the unstable minus end shrinks, if it is not stabilized by capping proteins. Treadmilling is a net addition of tubulin heterodimers at the plus end and the balanced net loss from the minus end.

MT-associated proteins (MAPs) are attached to MTs in vivo and play a role in their nucleation, growth, shrinkage, stabilization and motion. Of the MAPs, the tau family proteins have received special attention as they are involved in the pathophysiology of Alzheimer’s disease.

MTs are stabilized at their minus ends by the ► **centrosome** (also called microtubule organizing center, MTOC). Centrosomes are protein complexes containing among other proteins two centrioles (ring-like structures) and α -tubulin. Centrosomes serve as nucleation points for microtubular polymerization and constrain the lattice structure of an MT to 13 protofila-



Cytoskeleton. Figure 1 Cytoskeleton – see text for further explanation.

ments. Centrosome-independent nucleation is possible and may be induced by DNA or proteins such as cadherin. At the plus end, capping proteins such as mDIA can stabilize MTs. Stable MTs often are acetylated at lysine-40 of α -tubulin and detyrosinated at the C-terminal of the β -tubulin.

► **Motor proteins** move along MTs in an ATP-dependent manner. Members of the superfamily of kinesin motors move only to the plus ends and dynein motors only to the minus ends. The respective motor domains are linked via adaptor proteins to their cargoes. The binding activity of the motors to MTs is regulated by kinases and phosphatases. When motors are immobilized at their cargo-binding area, they can move MTs.

MTs extend from the centrosome throughout the cytoplasm to the plasma membrane, where they are stabilized by caps. Sliding along the MTs, kinesin and dynein motors transport their cargoes between the center and the periphery of the cell. MTs present in the axons of neurons are extended not only by addition of heterodimers to the plus ends but also by use of short MTs that initiate in the centrosome. Their axonal transport is mediated by dynein motors that are passively moved along actin filaments. Once formed in the axon, MTs serve as tracks for the fast axonal transport, i.e. the movement of membranous organelles and membrane proteins to the nerve ending.

Functions of MTs

Some specialized eukaryotic cells have cilia that show a whiplike motion. Sperm cells move with one flagella, which is much longer than a cilium but has a nearly identical internal structure called axoneme. It is composed of nine doublet MTs that form a ring around a pair of single MTs. Numerous proteins bind to the MTs. Ciliary dynein motors generate the force by which MTs slide along each other to cause the bending of the axoneme necessary for motion.

The membrane tubules and lamellae of the endoplasmic reticulum (ER) are extended in the cell with the use of MTs and actin filaments. Kinesin motors are required for stretching out the ER, whereas depolymerization of microtubules causes the retraction of the ER to the cell centre in an actin-dependent manner. Newly synthesized proteins in the ER are moved by dynein motors along MTs to the Golgi complex (GC), where they are modified and packaged. The resulting vesicles move along the MTs to the cell periphery transported by kinesin motors. MTs determine the shape and the position also of the GC. Their depolymerization causes the fragmentation and dispersal of the GC. Dynein motors are required to rebuild the GC.

When cells enter mitosis, the interphase array of MTs is dismantled. The centrosome duplicates, and the daughters move to opposite poles of the nucleus. After disassembly of the nuclear envelope, MTs emanat-

ing from both centrosomes show pronounced dynamic instability. They grow and shorten and thus probe the cytoplasm until they find attachment sites at the condensed chromosomes, the ► **kinetochores**. Dynein motors are involved in the attachment and in the subsequent segregation of the two sets of chromosomes. In some cells, spindles are formed in the absence of centrosomes. Here, microtubular nucleation is initiated at the kinetochores by DNA itself with the help of the GTPase Ran and/or the microtubule-destabilizing factor stathmin. Kinesin and dynein motors then bundle and focus the MTs at their minus ends [1].

Organization of Actin Filaments (F-actin)

Globular actin (► **G-actin**) has a molecular weight of about 42 kDa. In higher vertebrates, the 6 isotypes are expressed in a cell-specific manner, which contain 374/375 residues. They are present in striated muscle cells (skeletal and cardiac isotypes), smooth muscle cells (vascular and visceral isotypes) and in non-muscle cells such as neurons and glial cells (2 isotypes). All isotypes are N-terminally acetylated and bind ATP/ADP. F-actin is a double-stranded, right-handed helix with 14 actin molecules per strand and turn. F-actin has a diameter of 8 nm and is polarized with a pointed (minus) and a barbed (plus) end. The length of F-actin is regulated by dynamic instability and treadmilling (Figure 1b). Within the F-actin polymer the ATP of an actin protomer is hydrolyzed to ADP. Once the inorganic phosphate is dissociated from the actin-ADP complex, the actin molecule will ultimately dissociate from the pointed end. After exchange of ADP to ATP, G-actin binds to the protein profilin and is ready for the next cycle (Fig. 1c).

Numerous ► **actin binding proteins** (ABPs) influence the turnover of F-actin. They are regulated in their activity via membrane receptors and thus transmit extracellular stimuli to the actin cytoskeleton. The ► **small GTPases** of the Rho family RhoA, Rac1 and Cdc42 are important mediators in the signal transduction pathway (Fig. 1c). Upon stimulation they activate proteins of the WASP/Scar family that bind to and activate the Arp2/3 complex. Subsequently ATP-G-actins bound to profilin are nucleated, i.e. two ATP-binding G-actins self-assemble side by side and further G-actins are added in a head-to-tail fashion (Fig. 1c). Alternatively, branches may be formed on existing actin filaments. Capping proteins that bind to the barbed end can block further elongation. Proteins of the ADF/cofilin family can sever and depolymerize ADP actin filaments.

Functions of F-actin

The interaction with myosin motors enables F-actin to transport molecules as well as to change or maintain the shape of the cell by exerting tension. Thus, myosin-I motors move to the barbed end and can transport cargoes such as vesicles. When immobilized at the cargo site

they can move actin filaments. Two myosin-II motors can assemble in an antiparallel manner. Attached to antiparallel F-actin, they can slide the actin filaments over each other and thus cause contractions. Such actomyosin interactions are essential for skeletal muscle contraction. Moreover, the contractile ring formed in the telophase of the cell cycle is an actomyosin structure that separates the filial cells during cytokinesis. In addition, actomyosin interactions play critical roles in the regulation of the morphology, adhesion and migration of non-muscle cells. Obviously, drugs that destroy F-actin have profound effects on cell shape and proliferation. In addition, F-actin plays an important role in cell adherence and motility.

Drugs

Drugs with Actions on MTs

Numerous drugs bind to β -tubulin and thus affect the function of MTs. The alkaloids vinblastine and vincristine extracted from the plant *Catharanthus roseus* (also called *Vinca rosea*) and the semisynthetic derivatives vindesine, vinorelbine, and vinflunine can block mitosis and are clinically used for cancer treatment. When used at micromolar concentrations, the alkaloids bind with 1:1 stoichiometry to β -tubulin in a region between residues 175 and 213. The bound disassembled tubulin heterodimers can no longer polymerize to MTs and form paracrystalline tubulin-*Vinca* alkaloid arrays. Pre-existent MTs are subsequently depolymerized. Chromosomes are no longer separated but dispersed or clumped in the cytoplasm. The cells undergo apoptosis. When used at nanomolar concentrations, *Vinca* alkaloids may block mitosis at the metaphase-anaphase transition by inhibiting the dynamic instability and treadmilling of MTs, without affecting microtubular mass [2]. The cytostatic agent estramustin has similar effects. It blocks heterodimer polymerization at micromolar concentrations and inhibits dynamic instability at nanomolar concentrations.

In contrast, paclitaxel (isolated from the bark of the yew tree *Taxus brevifolia*) and its more potent analogue docetaxel (from *Taxus bacca*) bind to microtubules when used at nanomolar concentrations. By attaching to β -tubulin between amino acids 239 and 254, the agents block the release of heterodimers from MTs and prevent the dynamic instability necessary for the capturing of chromosomes without affecting microtubular mass. Both taxols are used for cancer treatment. There is evidence that the β -tubulin isotypes differ in their sensitivity to taxols. Thus, the upregulation of β -tubulin III expression in glioma as well as in lung and prostate cancer cells correlates with the resistance to taxols [3]. Their severe side effects include neurotoxicity, neutropenia, cardiac effects and immunosuppression. Taxols can lose their cellular activity due to the multi-drug resistance P-glycoprotein transporter. In addition, their aqueous insolubility makes

their application difficult. Therefore, several new compounds are currently under investigation [4].

The epothilones A and B are produced by the myxobacterium *Sorangia cellulosum*. They compete with taxols for microtubular binding sites, but in contrast to taxols are not substrates of the P-glycoprotein transporter. Discodermolide produced by the sponge *Discodermia dissoluta* competes with taxols for microtubular binding sites but has different properties. Unlike taxols and epothilones, it is active in cells with β -tubulin mutations. In addition, it has pronounced synergistic effects with taxols. It is active in cells that express the P-glycoprotein transporter. Further products investigated for their taxol-like effects are laulimalide and isolaulimalide from the sponge *Cacospongia mycofijiensis* and eleutherobin from the soft coral *Eleutherobia* [4].

In addition, the alkaloid colchicine (from *Colchicum autumnale*) blocks tubulin polymerization by binding to heterodimeric β -tubulin between amino acids 239 and 254. Since it inhibits the MT-dependent migration of granulocytes into areas of inflammation and their MT-dependent release of proinflammatory agents, it is used to treat attacks of gout. Its antimitotic effect in the gastrointestinal system induces diarrhoea. Nocodazole competes for the binding site of colchicine and has similar effects on heterodimeric β -tubulin.

In view of the importance of the motor dynein for microtubular function, this protein is currently considered as a new target for the development of cytostatic agents.

Drugs with Actions on F-actin

Several agents affect the turnover of F-actin. They are not used therapeutically but serve as experimental tools to study the role of F-actin in cell function.

The ►**cytochalasins** A, B, C, D, E, and H are found in various species of mould. Mainly cytochalasin B and D are used as experimental tools. Cytochalasin D is 10 times more potent, acting at concentrations between 2 and 35 nM in cell-free systems. Cytochalasins bind to the barbed end of F-actin and block the addition as well as dissociation of G-actin at that end. At micromolar concentrations, cytochalasin D can bind to G-actin and actin dimers and thus block additional polymerization. When applied to cultured cells, micromolar concentrations of cytochalasins remove stress fibres and other F-actin structures.

Swinholide A, isolated from the marine sponge *Theonella swinhoei*, sequesters actin dimers and induces their formation. One molecule of swinholide A binds to one dimer. In addition, swinholide A can sever F-actin by binding to the neighbouring protomers. Increased depolymerization of F-actin has also been reported.

Several toxins produced by marine sponges cause the destabilization of F-actin. They contain a macrocyclic ring and an aliphatic chain, by which they bind to actin protomers. The toxins that include reidispongioides,

sphinxolides, aplyronins, mycalolides, ulapualides, halichondramides and kabiramides are currently investigated for their potential use in cancer treatment [5].

Latrunculins A and B are macrolides from the sponge *Latrunculia magnifica*. Latrunculin A (≥ 50 nM) binds close to the nucleotide binding site of G-actin and blocks the assembly with F-actin without promoting disassembly.

Phalloidin and phalloidin are cyclic peptides from the mushroom *Amanita phalloides* that stabilize F-actin. Phalloidin binds to residues 114–118 of an actin protomere and blocks nucleotide exchange without interfering with nucleotide hydrolysis. It enhances the rate of nucleation as well as that of elongation. It slowly penetrates the cell membrane and is used for immunocytochemical localization of F-actin.

In contrast, jasplakinolide, a cyclodepsipeptide from the marine sponge *Jaspis johnstoni*, rapidly penetrates the cell membrane. It competes with phalloidin for F-actin binding and has a dissociation constant of approximately 15 nM. It induces actin polymerization and stabilizes pre-existing actin filaments. Dolastatin 11, a depsipeptide from the mollusk *Dolabella auricularia*, induces F-actin polymerization. Its binding site differs from that of phalloidin or jasplakinolide.

► Small GTPases

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Cytostatic Drugs

► Antineoplastic Agents

Cytotoxic Agents

► Antineoplastic Agents

Cytotoxic T-cells

Cytotoxic T-cells, or cytolytic T-cells (CTLs), are effector cells of the immune system that carry the CD8⁺ surface marker. These cells are able to recognize autologous cells expressing foreign antigens (e.g., virus infected cells). Recognition is depending on the recognition of the MHC-I molecule by the T-cell receptor present on CTLs. This interaction of CTLs and target cells can lead to the lysis of the target cell.

► Immune Defense

► T Cell Receptors

► DNA Vaccination and Genetic Vaccination

Danaparoid (Heparinoid)

► Anticoagulants

Databank

In the biosciences, a databank (or data bank) is a structured set of raw data, most notably DNA sequences from sequencing projects (e.g. the EMBL and GenBank databases).

► Bioinformatics

Database

A database (or data base) is a collection of data that is organised so that its contents can easily be accessed, managed, and modified by a computer. The most prevalent type of database is the relational database which organises the data in tables; multiple relations can be mathematically defined between the rows and columns of each table to yield the desired information. An object-oriented database stores data in the form of objects which are organised in hierarchical classes that may inherit properties from classes higher in the tree structure.

In the biosciences, a database is a curated repository of raw data containing annotations, further analysis, and links to other databases. Examples of databases are the SWISSPROT database for annotated protein sequences or the FlyBase database of genetic and molecular data for *Drosophila melanogaster*.

► Bioinformatics

Death Domain and Death Receptors

Extrinsic apoptosis signaling is mediated by so-called *death receptors*. Besides Tumor Necrosis Factor receptor type I (TNFR1) the group of death receptors comprises seven additional members (Fas, DR3 [death receptor 3], DR4 [TRAIL-R1 {TNF-related apoptosis-inducing ligand receptor 1}], DR5 [TRAIL-R2], DR6, p75-NGFR [p75-nerve growth factor receptor], and EDAR [ectodermal dysplasia receptor]). Death receptors belong to the tumor necrosis factor receptor gene superfamily. Signaling of these death receptors is mediated by their cytoplasmic part containing a conserved sequence of approximately 90 amino acids length termed *death domain* (DD) (► [Death Domain Superfamily](#)). Adapter molecules, also possessing a DD, like FADD or TRADD, can be recruited to these receptors by homophilic DD/DD interactions, thereby initiating caspase activation and apoptotic signals.

► Tumor Necrosis Factor (TNF)

► Apoptosis

► Caspases

Death Domain Superfamily

Death domain (DD) superfamily consists of structurally related homotypic interaction motifs of approximately 90 amino acids. The motifs are organized in six antiparallel amphipathic α -helices, the so-called DD fold. The four members of the superfamily are the death domain (DD), the death effector domain (DED), the caspase activation and recruitment domain (CARD), and the Pyrin domain. All are important mediators for the assembly of caspase activating complexes.

► Tumor Necrosis Factor (TNF)

► Apoptosis

► Caspases

Defensins

Definition

Defensins are a group of antimicrobial and cytotoxic peptides made by immune cells. There are seven defensins in humans, six alpha-defensins and one beta-defensin, which are involved in the innate immune defense at the surface of epithelia from the respiratory tract, the intestinal tract or the urinary tract.

- Immune Defense
- Nuclear Factor Kappa B

Delayed-rectifier K⁺ Channels

Delayed-rectifier K⁺ channels activate with a delay and mediate outwardly-rectifying K⁺ currents. These channels may make a significant contribution to the repolarizing phase of nervous action potentials.

- K⁺ Channels

Delayed Type Hypersensitivity Reaction

Delayed type hypersensitivity (DTH) reactions (synonym type IV allergic reactions) are exaggerated, T-lymphocyte mediated, cellular immune reactions to foreign substances, which require one to two days to manifest clinical symptoms.

- Allergy

Dendritic Cells

Dendritic cells (DC) are important antigen-presenting cells mostly derived from the bone marrow. Microscopically they show characteristic long cytoplasmic extensions called “dendrites.” There are several types of specialized DC present in all lymphoid tissues and major

organs, in particular in the skin where they are also called Langerhans Cells. Another specialized population called plasmacytoid DC is found in the marginal zone of the spleen and represents the major IFN- α producing cell type in the organism. DC are constantly sensing their environment for antigenic material derived from pathogenic microorganisms or allergens with the help of DC-specific pattern recognition receptors such as Toll-like receptors. Following antigen uptake and activation, DC migrate to lymphoid organs to present processed antigen to T- and B-lymphocytes.

- Immue Defense

Dependence

Dependence is a somatic state which develops after chronic administration of certain drugs. This condition is characterized by the necessity to continue administration of the drug to avoid the appearance of withdrawal symptoms. Withdrawal symptoms are relieved by the administration of the drug upon which the body was “dependent”. Psychological dependence is due to (e.g., social) reinforcement processes in the maintenance of drug-seeking behavior.

- Drug Addiction/Dependence

Depolarisation

A reduction in charge separation across the plasma membrane resulting in a less negative membrane potential. For example, a change from a resting potential of -60 to -40 mV. The membrane potential is always measured as the charge on the inside of the cell relative to outside, and has a negative value at rest reflecting the unequal distribution of charged species (small ions and charged molecules including proteins) across the membrane. The summation of temporally and spatially related depolarisations can reach a ‘threshold’ value that triggers the opening of voltage-dependent ion channels, producing a rapid, large ‘all or none’ depolarisation called an action potential. In skeletal muscle cells this results in muscle contraction.

- Nicotinic Receptors
- Voltage-dependent Na⁺ Channels
- Non-selective Cation Channels

Depolarization-induced Ca^{2+} Release

► DICR

Depression

► Antidepressants

Dermatomycoses

Dermatomycosis is an illness of the skin caused by fungi.

► Antifungal Drugs

Dermatophytes

Dermatophytes are a special type of fungi, invading the skin and able to grow on keratin as a sole nutrient base.

► Antifungal Drugs

Descending Inhibitory Pathway

The descending inhibitory pathway is an endogenous pain-suppressing system, which becomes activated especially under pathological conditions, when stress is present. A key part of this system is an area of the midbrain, called the “►periaqueductal grey” (PAG). The PAG is rich in enkephalin-containing neurons. It receives inputs from many other brain regions, including the hypothalamus, cortex and thalamus. These connections may represent the mechanism whereby cortical and other inputs control pain perception. Activation of PAG neurons leads to the activation

of neurons in the rostroventral medulla, which includes the nucleus raphe magnus. From the nucleus raphe magnus, serotonin- and enkephalin-containing neurons project to the substantia gelatinosa of the dorsal horn of the spinal cord and exert their inhibitory influences on nociceptive transmission. There is also a noradrenergic pathway, which originates from the locus ceruleus, which also has an inhibitory effect on the nociceptive transmission in the dorsal horn. The PAG and the substantia gelatinosa are especially rich in enkephalin-containing neurons, suggesting that opioid peptides function as transmitters in this system. The antinociceptive effects of opioids are believed to be at least partly mediated by effects on the descending inhibitory pathway.

► Nociception

Desensitised State

A condition in which a receptor is unresponsive despite the presence of agonist; also referred to as a ‘refractory state’. Typically this state is the consequence of prolonged exposure to agonist, and occurs after receptor activation; it is a built in mechanism to limit a receptor’s effects. Mechanistically the desensitised state differs from the resting, closed state of a receptor because in the latter state, a receptor *can* respond to agonist. This difference predicts that these states are structurally distinct. The desensitised state may also be stabilised by very low concentrations of agonist, such that no measurable activation of the receptor precedes it. Desensitisation is an intrinsic property of many receptors but can also be influenced by other interactions or modifications, such as phosphorylation.

- Tolerance and Desensitization
- G-protein-coupled Receptors
- Glycine Receptors
- Ionotropic Glutamate Receptors
- Nicotinic Receptors
- Neurotransmitter Transporters

Desensitization

Desensitization is the rapid attenuation of receptor activation as a result of stimulation of cells and occurs in seconds to minutes. Receptor phosphorylation

by G-protein-coupled receptor kinases and second-messenger-regulated kinases as well as receptor/G-protein uncoupling contribute to this process. In the continued presence or at high concentrations of agonistic ligands, ligand gated ion channels may undergo desensitization by entering a permanently closed state. While the ligand binding domain is occupied by the agonist, the desensitized channel is unable to re-open. For ligand-gated ion channels, the structural basis of desensitization is not understood. For voltage-gated K^+ channels, the 'ball and chain' model suggests a mechanism of ion channel desensitization.

- Tolerance and Desensitization
- G-protein-coupled Receptors
- Glycine Receptors
- Ionotropic Glutamate Receptors
- Nicotinic Receptors
- Neurotransmitter Transporters
- Voltage-gated K^+ Channels

Desmoplakin

Desmoplakin is the most abundant desmosomal component that plays a critical role in linking intermediate filament networks to the desmosomal plaque. Desmoplakin forms rod-like dimers that bind to intermediate filaments and to the cadherin-associated proteins plakoglobin and plakophilin. Gene knock-out experiments have revealed an essential role of desmoplakin in establishing cell–cell contacts in early mouse embryos.

- Cadherins/Catenins
- Cytoskeleton

Deubiquitinating Enzymes (DUBs)

The human genome contains more than 90 different DUBs. Besides cleaving ubiquitin from distinct substrates, DUBs are also responsible for the recycling of free ubiquitin from ubiquitin chains and processing of ubiquitin- or ubiquitin like precursor proteins. Certain DUBs are also associated with the proteasome in order to detach ubiquitin chains before proteolysis.

- Ubiquitin/Proteasome

Dexamethasone

Synthetic steroid with strong glucocorticoid-agonistic activity. Dexamethasone is over 10 times more potent than cortisol due to a higher binding affinity for glucocorticoid receptor and a decreased clearance rate of the compound. Due to its potency, dexamethasone is widely used in the clinics for the treatment of inflammatory diseases.

- Gluco-mineralocorticoid Receptors

DHPR

- Dihydropyridine Receptor

Diabetes

Obese people are predisposed to diabetes and cardiovascular disease. The term diabetes (sometimes referred to as 'syndrome X' or 'metabolic syndrome') reflects the intricate association between these disorders.

- Diabetes Mellitus

Diabetes Insipidus

A disease where the kidney is unable to concentrate urine, characterized by hyposthenuria, polyuria and polydipsia. There are two major forms of diabetes insipidus: central (caused by vasopressin deficiency) and nephrogenic (caused by resistance of the kidney towards vasopressin). This type of diabetes is not related to diabetes mellitus (insulin deficiency or resistance), where also large amounts of urine and glucose are excreted by the kidney. Other types of diabetes insipidus are the dipsogenic (neurological origin) and gestational diabetes insipidus (during pregnancy).

- Vasopressin/Oxytocin

Diabetes Mellitus

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Synonyms

Diabetes mellitus

Definition

Diabetes mellitus is defined as hyperglycaemia (fasting > 7 mM and/or 2 h postprandial > 11.1 mM) due to absolute or relative lack of insulin. The most common forms are type 1 diabetes (prevalence 0.25%), with absolute lack of insulin, and type 2 diabetes (prevalence 4–6%) which is due to the combination of insulin resistance and insufficient insulin secretion.

Basic Mechanisms

Type 1 Diabetes (Previously Insulin-Dependent Diabetes Mellitus, IDDM, or Juvenile Diabetes)

Progradient destruction of insulin-secreting β -cells by an autoimmune mechanism during a period of several years [1]. The diagnosis is confirmed by detection of antibodies against β -cell proteins (glutamic acid decarboxylase, tyrosine phosphatase IA-2, insulin). Islet cell destruction is probably mediated by activated T lymphocytes. There is a genetic susceptibility to type 1 diabetes, and several predisposing loci (e.g. the MHC locus) and alleles of genes associated with the disease risk (the insulin gene, PTPN22, CTLA-4) have been identified [1]. In addition, genetic and epidemiological data indicate a strong environmental factor in the pathogenesis.

Type 2 Diabetes (Previously non-Insulin-Dependent Diabetes Mellitus, NIDDM)

Type 2 diabetes is a frequent consequence of the ►metabolic syndrome (obesity, insulin resistance, dyslipidemia, hypertension). Obesity causes insulin resistance which is initially compensated by hyperinsulinemia. In the course of insulin resistance, the β -cell function deteriorates and insulin secretion is reduced, leading to relative insulinopenia. In particular, the initial response (first phase) of the pancreas to a glucose load is impaired during the first stages of the disease. Fasting hyperglycaemia is preceded by a variable period of impaired glucose tolerance (IGT) during which secondary complications (micro- and macrovascular) start to develop. Thus, in many type 2 diabetics, secondary complication can be diagnosed at the time of the detection of hyperglycaemia; these are accelerated and aggravated by other components of the metabolic syndrome (dyslipidemia, hypertension).

Obesity and insulin resistance appear to be due to a heterogenous combination of genes involved in the control of appetite and thermogenesis. Several genes have been identified that are involved in the regulation of food intake and of body weight (e.g. leptin, the leptin receptor, melanocortin receptors; see chapter on appetite control). However, the genes responsible for the polygenic obesity are largely unknown. Data obtained from rodent models indicate that islet cell failure is produced by a different set of genes; obesity is a necessary condition for their effects on the pancreas. This interaction of two different genetic constellations (obesity and diabetes genes) explains the heterogeneity of the type 2 diabetes with regard to its association with body weight.

The molecular pathology of the β -cell destruction in the course of insulin resistance is largely unknown. It has been suggested that the constant hyperstimulation of the β -cell by glucose ('glucose toxicity') or elevated fatty acids ('lipotoxicity') may lead to cell damage.

Pharmacological Intervention

Type 1 diabetes always requires treatment with insulin. The preferred therapeutic regimen is the 'intensified conventional therapy' (ICT) which combines 1–2 daily injections of a retarded insulin (usually NPH insulin) with 3–4 injections of a short-acting insulin (e.g. regular insulin) at mealtimes. ICT requires 4–5 daily blood sugar determinations and adjustment of the dose to (preprandial) blood sugar and (estimated) carbohydrate content of meals. If the complicated ICT regimen is not feasible, a conventional regimen (CT) may be employed which comprises two daily injections of a combination of NPH with regular insulin (two thirds of the daily dose before breakfast, one third before dinner). CT usually applies constant doses of insulin and requires a stricter adherence to the diet than ICT, i.e. meals that contain a controlled carbohydrate content and are timed according to the profile of the insulin injections.

Insulin preparations (Table 1): Recombinant human insulin obtained from genetically engineered *E. coli* or yeast is used predominantly. Retarded preparations are generated by addition of protamine (*NPH insulin*, neutral protamine Hagedorn) which may provide the 'basal' supply in both ICT and CT. Five insulin analogs with altered amino acid sequence and different pharmacokinetic characteristics are available: *insulin lispro* (swap of B28 proline and B29 lysine), *insulin aspart* (exchange of aspartate for B28 proline), and *insulin glulisine* (exchange of lysine for asparagine B3 and glutamate for lysine B29) exhibit a more rapid onset and a shorter duration of action because of a faster dissociation of the hexameric insulin–zinc–phenol complex. *Insulin glargine* is a long-acting analogue which is soluble at low pH because of two additional arginines

Diabetes Mellitus. Table 1 Pharmacokinetic characteristics of the most commonly used insulin preparations and analogs

	onset of action	maximum	duration of action
<i>short acting insulins:</i>			
insulin lispro	15 min	½–1 h	2–5 h
insulin aspart	15 min	½–1 h	2–5 h
insulin glulisine	15 min	½–1 h	2–5 h
regular insulin	30 min	1–2 h	5–8 h
<i>long acting insulins:</i>			
NPH insulin	30–60 min	2 h	11–20 h
insulin glargine	60 min	4 h	12–30 h
insulin detemir	60 min	5–8 h	12–22 h

at the C terminus of the B chain. After injection, the analogue precipitates from its solution and forms a long-acting depot with a steady absorption kinetic. *Insulin detemir* lacks threonine B30 and is myristoylated at lysine B29. Binding of the analogue to serum albumin is responsible for its longer duration of action which is comparable to that of *insulin glargine*.

Type 2 diabetes: After clinical diagnosis, weight normalization by hypocaloric, low-fat diet and exercise may normalize glucose homeostasis for a period of several years. Accordingly, this intervention markedly delays the onset of overt hyperglycaemia when employed at the stage of IGT. Because the ‘lifestyle changes’ are difficult to maintain, and because of the progression of the islet cell failure, pharmacological intervention will usually become inevitable and has to be intensified throughout the course of the disease. Initially, normalization of blood sugar can be accomplished with oral antidiabetics. Later on, a combination of oral antidiabetics with insulin or insulin alone (preferably ICT) will be required.

β-Cytotropic agents: These agents enhance the effect of glucose on the secretion of insulin [2]. Commonly used drugs are sulfonylurea derivatives and the more recently introduced benzoic acid derivatives ► **repaglinide** and **nateglinide**. Their receptor is a membrane protein (SUR, ► **sulfonylurea receptor**) which regulates the activity of the ATP-dependent potassium channel of the pancreatic β-cell (Kir 6.2). Binding of ATP, or of a sulfonylurea, to SUR reduces the potassium current and causes membrane depolarization, subsequently opening of voltage-gated calcium channels, and calcium-stimulated exocytosis of insulin-containing granules (Fig. 1). Lack-of-function mutants of SUR are characterized by an increased secretion of insulin (congenital hypoglycaemia). The effect of the β-cytotropic agents on the channel is antagonized by the ‘potassium channel openers’ diazoxide and minoxidil.

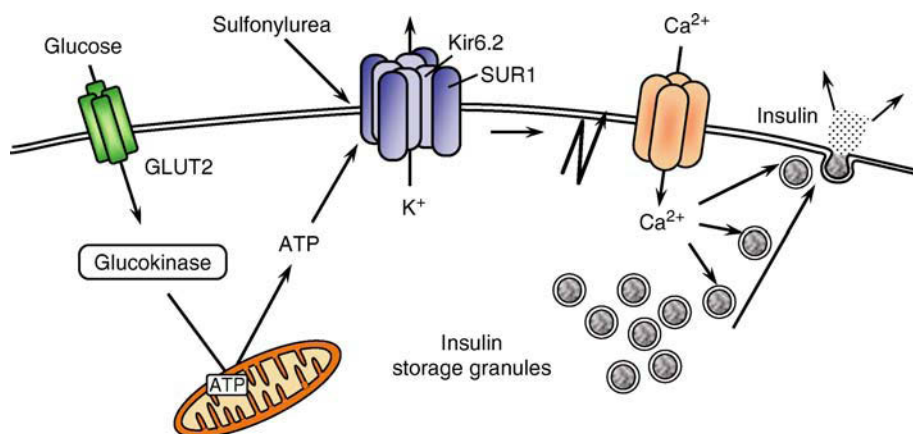
Two isotypes of SUR have been cloned, SUR1 and SUR2. In addition, two splicing variants of SUR2, distinguished by 42 C-terminal amino acids, have been

identified. SUR1 is mainly expressed in β-cells. SUR2B is expressed in vascular smooth muscle, heart, skeletal muscle and brain, whereas expression of SUR2A is restricted to heart and skeletal muscle. Sulfonylureas exhibit a higher affinity for the SUR1. Block of SUR2 by minoxidil or diazoxide produces vasodilation; stimulation of SUR2 by high, non-therapeutic concentrations of glibenclamide produces vasoconstriction. A potential effect of sulfonylureas on the cardiac SUR2, producing cardiac arrhythmia in particular under ischemic conditions, is a matter of ongoing debate.

To date, the commonly used sulfonylureas are the long-acting derivatives glibenclamide (US synonym: glyburide) and glimepiride. Glimepiride is believed to have a somewhat longer duration of action than glibenclamide, and to exert additional extrapancreatic effects. The mechanism of these potential extrapancreatic effects is not defined, and their clinical relevance is unclear. The non-sulfonylurea potassium channel blockers repaglinide and nateglinide have been shown to act by the same mechanism of action as the sulfonylureas. The duration of action of repaglinide and nateglinide is short because of their rapid metabolism and/or excretion. These pharmacokinetic parameters are thought to allow a specific reduction of postprandial glucose excursions. However, because the effects of the potassium channel openers largely depend on plasma glucose levels, both long- and short-acting agents mainly stimulate a postprandial insulin secretion. A potential advantage of the short-acting derivatives might be a reduced risk of hypoglycaemia.

Exenatide: ► **Exenatide** is a peptide which mimics the effect of glucagon-like peptide 1 (GLP-1) in stimulating glucose-dependent insulin secretion from β-cells and inhibiting glucagon secretion from α-cells [3]. Exenatide is injected subcutaneously and improves glycaemic control in type 2 diabetics and causes a significant reduction of body weight.

DPP-IV inhibitors: Inhibition of the dipeptidyl peptidase IV (by ► **sitagliptin** or ► **vildagliptin**) prolongs the duration of action of endogenous GLP-1 and thereby



Diabetes Mellitus. Figure 1 Mechanism of insulin secretion and its stimulation by β -cytotropic agents. Glucose enters the β -cell via GLUT2, is phosphorylated by glucokinase, and generates ATP. Rise in the ATP-ADP ratio results in closure of ATP-dependent K⁺ channels, membrane depolarization and opening of voltage-gated Ca²⁺ channels. Ca²⁺ influx activates the exocytosis of insulin-containing secretory granules. The ATP-dependent K⁺ channel consists of Kir6.2 (a K⁺ inward rectifier) and SUR (sulfonyleurea receptor). Sulfonyleureas block the ATP-dependent K⁺ channels by enhancing the effect of ATP.

improves glycaemic control, either alone or in combination with other antidiabetic agents [4].

Metformin: The biguanide ►metformin lowers blood sugar mainly by inhibition of glucose output from the liver. It is generally accepted that this effect reflects a marked inhibition of gluconeogenesis and lipogenesis, presumably mediated by a stimulation of the AMP-dependent kinase [5]. Other effects of metformin (stimulation of glucose transport in muscle, inhibition of intestinal glucose absorption) require higher concentrations and appear to contribute little to the in-vivo effect.

Under certain circumstances, and very rarely, the inhibition of gluconeogenesis by metformin may suppress lactic acid metabolism and precipitate a potentially fatal lactic acidosis. Impairment of renal function, liver disease, alcoholism, conditions that give rise to increased lactate production (e.g. congestive heart failure, infections) are therefore contraindications for the application of metformin.

Oral formulations of metformin are rapidly and completely absorbed. The agent is poorly bound to plasma proteins; its duration of action is determined exclusively by renal elimination. Higher concentrations of metformin than in most tissues are found in the intestinal mucosa, giving rise to common side effects (irritation, diarrhoea etc.).

Acarbose and Miglitol: These agents are specific inhibitors of intestinal glucosidases and reduce the conversion of sucrose and starch to glucose. Their main effect is a delay, not a complete inhibition, of the absorption of carbohydrates. Postprandial blood sugar excursions are effectively reduced. Because a small portion of the carbohydrates enters the colon, their microbial degradation frequently causes flatulence or

meteorism. Acarbose produces a somewhat lower reduction of HbA_{1c} levels than glibenclamide and metformin, but may enhance the effect of these agents. When given to patients with IGT, acarbose reduces the progression of the disease to overt hyperglycaemia by 30%.

Thiazolidinediones (PPAR γ -agonists): Thiazolidinediones (►pioglitazone, ►rosiglitazone) lower blood glucose levels in animal models of insulin resistance and also in insulin resistant patients. They are agonists of the ►peroxisome proliferator-activated receptor γ (PPAR γ). Because they enhance the effect of insulin and reduce serum insulin levels in insulin resistant patients, thiazolidinediones are usually referred to as 'insulin sensitizers'.

PPAR γ is a transcription factor which controls the expression of enzymes and proteins involved in fat and glucose metabolism. More importantly, stimulation of this receptor induces differentiation of preadipocytes to adipose cells. It is believed that the formation of additional, small fat cells lowers free fatty acids and hepatic triglycerides, thereby correcting insulin resistance.

- Metabolic Syndrome
- Insulin Receptor
- Glucose Transporters
- ATP-dependent K⁺ Channel
- Oral Antidiabetic Drugs

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ryanodine receptor (RyR1), resulting in Ca^{2+} release through RyR1. Ca^{2+} -induced Ca^{2+} release (CICR) in the skeletal muscle is too slow to make a significant contribution to DICR. In cardiac muscle, in contrast, Ca^{2+} influx through the α 1C subunit of DHPR triggers CICR. Consistently, contraction of skeletal muscle is independent of the external Ca^{2+} , whereas cardiac muscle contraction is dependent on it.

- Ryanodine Receptor
- Voltage-dependent Ca^{2+} Channels

Diacylglycerol

Diacylglycerol is glycerol esterified to two fatty acids at the sn-1 and sn-2 positions. It is a membrane-embedded product of phospholipase C action and an activator of protein kinase C. It is also an intermediate in the biosynthesis of triacylglycerol, phosphatidylethanolamine and phosphatidylcholine.

- Phospholipases
- Protein Kinase C

Dicer

Dicer represents the key enzyme in the RNAi pathway. Dicer is also known as Helicase with RNase motif, heRNA, Helicase-moi, K12H4.8-like, or KIAA0928. Dicer produces cleaves long double-stranded RNA into small pieces of about 21–23 nucleotides. These so-called siRNA duplexes produced by the action of Dicer contain 5'-phosphates and free 3' -hydroxyl groups

- RNA Interference (RNAi) – siRNA

DICR

DICR (depolarization-induced Ca^{2+} release) is Ca^{2+} release triggered by depolarization of the sarcolemma. In skeletal muscle, conformational change in the voltage sensor (α 1S subunit of the dihydropyridine receptor) in the T-tubule is directly transmitted to the

Differential Display

Differential display is a method for identifying differentially expressed genes, using anchored oligo-dT, random oligonucleotide primers and polymerase chain reaction on reverse-transcribed RNA from different cell populations. The amplified complementary DNAs are displayed and comparisons are drawn between the different cell populations.

- Gene Expression Analysis
- Microarray Technology

Differentiation Factors

- Growth Factors

Digitalis

- Cardiac Glycosides

Dihydrofolate Reductase

Dihydrofolate reductase is required for the synthesis of tetrahydrofolate, a co-factor required for the transfer of single carbon groups. Inhibition of dihydrofolate

reductase blocks the synthesis of DNA and RNA (thymidylate and purines). The enzyme inhibitors include antibacterial and -parasitic drugs (pyrimethamine, trimethoprim) and the antineoplastic agent methotrexate.

- ▶ Folic Acid
- ▶ Vitamin B12
- ▶ Antineoplastic Agents
- ▶ Antiprotozoal Drugs

Dihydropyridine Receptor

Dihydropyridine receptor (DHPR) is a member of voltage-dependent Ca^{2+} channels (CaV_1 , L-type), which specifically binds to dihydropyridine derivatives, a group of the Ca^{2+} channel blockers. $\text{Ca}_v1.1$ works as the voltage sensor for skeletal muscle contraction, and $\text{Ca}_v1.2$, as Ca^{2+} -influx channel for cardiac muscle contraction.

- ▶ Voltage-dependent Ca^{2+} Channels
- ▶ Ca^{2+} Channel Blockers
- ▶ Ryanodine Receptor

(R)-(+)-N-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)- β -alanine

- ▶ Pantothenic Acid

L-Dihydroxyphenylalanine

- ▶ L-DOPA/Levodopa

4-Dihydroxyphenylethylamine

- ▶ Dopamine System

Dimeric Transcription Factors

Sequence-specific transcription factors often bind as multimers especially as dimers to DNA. This allows binding of mirror-imaged sequences (palindromes) in the DNA that are separated by a few spacer nucleotides. The dimerization is stabilized by hydrophobic motifs within dimerization motifs of each transcription factor molecule. Dependent on the nature of the dimerization domain and the abundance of individual transcription factors homo- or heterodimers can form and bind to palindromes with differential activity.

- ▶ Transcriptional Regulation

Dioxins

Dioxins are prominent members of the class of polychlorinated hydrocarbons that also includes dibenzofuran, biphenyls and others. Dioxins are highly toxic environmental contaminants. Like others small planar xenobiotics, some dioxins bind with high affinity to the arylhydrocarbon (Ah) receptor. Dioxins activate the receptor over a long time period, but are themselves poor substrates for the enzymes which are induced via the Ah-receptor. These properties of the dioxins and related xenobiotics may be important for the toxicity of these compounds. Dioxins like 2,3,7,8-tetrachloro-p-dibenzodioxin can cause persistent dermatosis, like chloracne and may have other neurotoxic, immunotoxic and carcinogenic effects.

- ▶ Arylhydrocarbon Receptor
- ▶ Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes

Dipeptidase

An exopeptidase that can only degrade a dipeptide. Examples are carnosine dipeptidase I (*MEROPS* M20.006), which degrades carnosine (β -Ala-His), and membrane dipeptidase (*MEROPS* M19.001), which is important in the catabolism of glutathione, degrading the dipeptides Cys-Gly. Dipeptidases are included in Enzyme Nomenclature sub-subclass 3.4.13.

- ▶ Non-viral Peptidases

Dipeptidylpeptidase

An exopeptidase that sequentially releases a dipeptide from the N-terminus of a protein or peptide. Dipeptidyl-peptidases are included in Enzyme Nomenclature subclass 3.4.14 along with tripeptidyl-peptidases.

► Non-viral Peptidases

Dipeptidylpeptidase IV

Dipeptidylpeptidase IV (also known as *CD26*) cleaves *neuropeptide Y*, and *peptide YY* to generate their subtype-selective fragments NPY_{3-36} and PYY_{3-36} .

► Non-viral Peptidases

► Neuropeptide Y

Direct Thrombin Inhibitors

► Anticoagulants

Discharge of Neurons

Discharge is the electrical signal of an activated neuron or a group of neurons. During a discharge or 'action potential', neurons lose their negative intracellular potential and become positively charged for a fraction of a second (1–4 milliseconds). This excitation is conducted to the nerve terminals which form synapses with other cells and can thereby facilitate action potential generation in these target cells (at excitatory synapses) or impede action potential generation (inhibitory synapses).

► Synaptic Transmission

► Antiepileptic Drugs

Disease-modifying Anti-rheumatic Drugs (DMARDs)

The term disease-modifying anti-rheumatic drug or DMARD refers to a category of pharmacological agent that has some ability to retard the rate of structural joint damage. This property distinguishes DMARDs from non-steroidal anti-inflammatory drugs which improve certain features of inflammation but have no capability to modify the rate of structural damage to joints in human disease, although they may do so in animal models. The term DMARD has also been applied to drugs that result in sustained improvement in physical function or a decrease in inflammatory synovitis.

► Rheumatoid Arthritis

► Gold Compounds

Dissociation Constant

The dissociation constant (K_d) is the concentration of free ligand that results in occupancy of 50% of the receptors for this ligand available in the system.

► Drug–Receptor Interaction

Dissociative Anesthetic

Dissociative anesthetic is a term applied to phencyclidine and ketamine which induce a peculiar subjective state of dissociation from the environment, together with sedation, immobility, amnesia, analgesia, and ultimately coma.

► Psychotomimetic Drugs

Distribution of Drugs

► Pharmacokinetics

Diuretics

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Synonyms

Natriuretics; Antinatriferics

Definition

Diuretics promote the urinary excretion of sodium and water by inhibiting the absorption of filtered fluid across the renal tubular epithelium. The ensuing reduction in Na reabsorption reduces the Na content of the body, the critical determinant of extracellular and plasma fluid volumes. Thus, the use of diuretics is primarily indicated in the treatment of edematous diseases and of arterial hypertension.

Mechanisms of Action

The mammalian kidney generates its excretory product, the urine, in a two-step process where filtration of a large volume of plasma-like fluid across the glomerular blood capillaries is followed by the reabsorption and secretion of solutes and water across the tubular epithelial cell layer. Transcellular reabsorption of filtered Na is achieved by apical Na uptake along a favorable electrochemical gradient and by basolateral Na extrusion through the energy consuming action of Na, K-ATPase. The renal tubule is cytologically heterogeneous along its longitudinal axis, and one expression of this heterogeneity is the type of transport protein responsible for apical Na uptake. As a general rule, currently available diuretics inhibit a specific apical Na transporter, and their action therefore displays tubule segment specificity.

Na uptake in the proximal tubule, a segment in which about 2/3 of the filtered Na is reabsorbed, is mediated by a Na^+/H^+ exchanger (NHE3) and a number of other transporters that typically carry a second solute in a Na-dependent cotransport mode. Along the thick ascending limb of the loop of Henle, Na uptake occurs mostly through the electroneutral $\text{Na}^+, \text{K}^+, 2\text{Cl}^-$ cotransporters (NKCC2) in a process that accounts for roughly 25% of total renal Na reabsorption. Na uptake in the distal convoluted tubule is mediated by a NaCl cotransporter (NCC), and this segment accounts for about 5% of Na reabsorption. Finally, electrogenic Na reabsorption through the epithelial Na^+ channel (ENaC) is the uptake mode across the cortical collecting duct, a process that may be responsible for 2–3% of Na reabsorption. Na transport through ENaC is regulated by the adrenal gland steroid aldosterone.

As a general rule, increases of renal blood flow and/or $\text{glomerular filtration rate}$ (GFR) correlate rather well with increased urinary excretion of solutes and water. The underlying causes for this correlation are not fully understood, but they reflect incomplete adjustments of tubular reabsorption to an increase of tubular electrolyte load.

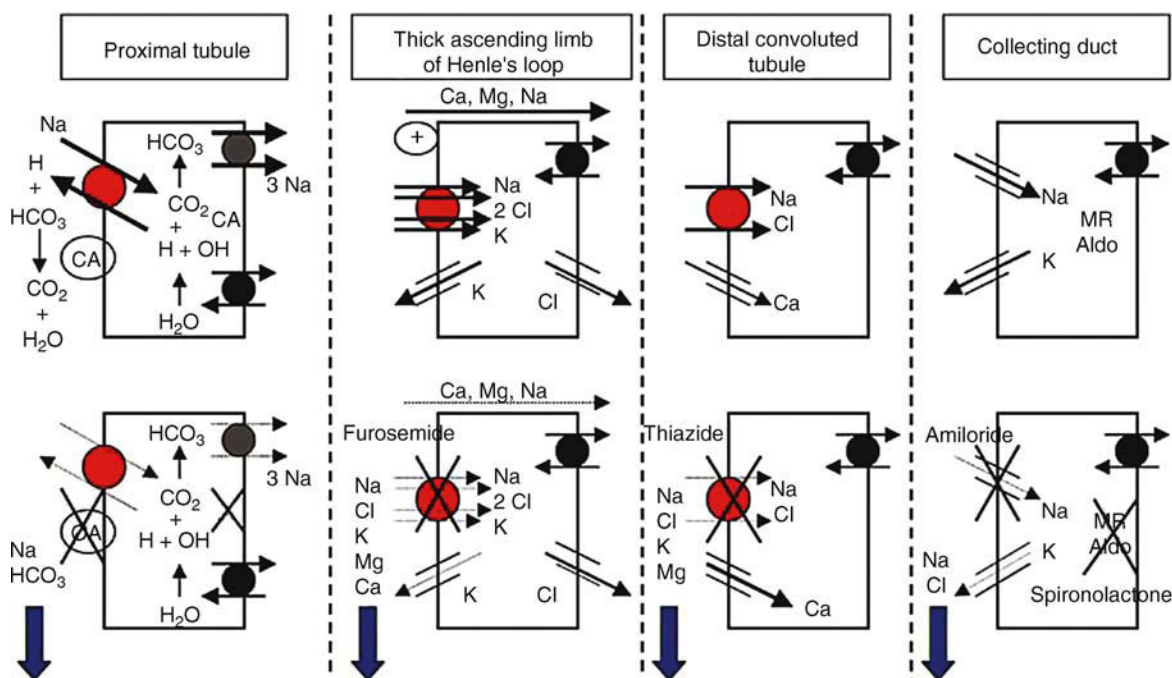
Proximal Tubule Diuretics

Inhibition of $\text{carbonic anhydrase}$ (CA) causes inhibition of NaHCO_3 reabsorption in the proximal tubule (Fig. 1). Apical Na uptake through NHE3 requires the delivery of H ions to the intracellular binding site of the transporter and of HCO_3^- to the basolateral $3\text{Na}/\text{HCO}_3^-$ cotransporter. The generation of H and HCO_3^- at appropriate rates from H_2O and CO_2 is dependent upon catalysis by intracellular carbonic anhydrase (type II CA). In addition, carbonic acid dehydration in the tubular lumen is catalyzed by a CA located in the luminal membrane of proximal tubules (type IV CA). Both enzymes must be functional for reabsorption of Na HCO_3 in the proximal tubule to proceed at normal rates. CA inhibitors (acetazolamide, benzolamide, methazolamide, and others) cause NaHCO_3 reabsorption to decrease by about 40–80% and urinary Na HCO_3 excretion to increase by about 25–30%. Efficacy of CA inhibitors is diminished by a consistent reduction of GFR.

Activation of dopamine 1 receptors (fenoldopam) or inhibition of A1 adenosine receptors (CVT 124, KW-3902) are additional approaches that cause increased NaCl excretion at least in part by inhibition of proximal reabsorption.

Loop of Henle Diuretics

The so-called loop diuretics (sulfonamide derivatives like furosemide, bumetanide, torsemide, and others, and phenoxyacetic acid derivatives like ethacrynic acid) augment the excretion of NaCl through inhibition of the electroneutral NKCC2 cotransporter in the apical membrane of the thick ascending limb (TAL) of the loop of Henle. Loop diuretics directly interact with the transport protein, presumably by binding to a region that is also critical for binding of a chloride ion. By collapsing the normally lumen positive transepithelial potential difference loop diuretics abolish the electrical driving force that is responsible for the paracellular absorption of Na and other cations such as Ca and Mg. Since NaCl transport across the TAL causes the accumulation of medullary solutes, loop diuretics lead to a dissipation of the $\text{cortico-medullary solute gradient}$, and thereby disable the urinary concentrating mechanism. Renal blood flow and glomerular filtration rate are usually not affected by loop diuretics. While some of the natriuretic action of loop diuretics is due to direct inhibition of NKCC2, another part is the secondary



Diuretics. Figure 1 Schematic representation of four cells representative for the proximal tubule, the thick ascending limb of the loop of Henle, the distal convoluted tubule, and the collecting duct. The upper diagrams show the normal functional state of these cells whereas the lower diagrams show the effect of the segment-specific diuretics. Circles indicate transport proteins, and double lines represent channels. Arrows indicate the normal direction of net transport, and the boldness of the arrows is a qualitative index of transport rate. Only those cellular features are shown that are relevant to understanding the action of the diuretic agents. CA, carbonic anhydrase; MR Aldo, mineralocorticoid receptor aldosterone complex.

consequence of simultaneous stimulation of PGE₂ production. Blockade of cyclooxygenases, particularly of **cyclooxygenase-2**, has been shown to reduce loop diuretic-induced natriuresis by about 50% through mechanisms that are not fully understood. Since PGE₂ generated by cyclooxygenase-2 under conditions of reduced NaCl transport is also a main factor in macula densa stimulation of renin secretion, loop diuretics are typically associated with an increase in renin secretion and greatly elevated plasma renin levels. Loop diuretics act from the luminal aspect of the tubule. Because of their intense protein binding these agents are not filtered effectively, and they gain access to the tubule lumen by secretion. Loss of function mutations in the NKCC2 gene are the cause of **Bartter syndrome** type I, and the symptoms of this disease are comparable to those caused by loop diuretics including salt and water loss, calciuria, magnesiuria, hypokalemia, metabolic alkalosis, hyperreninemia, and hyperprostaglandinuria.

Distal Convoluted Tubule Diuretics

Electroneutral NaCl transport in the distal convoluted tubule is inhibited by the class of thiazide diuretics (chlorothiazide, hydrochlorothiazide, metolazone, chlorthalidone, and others). Thiazides interfere with the Cl binding site of NCC, and cause a relatively small

increase in Na excretion. Inhibition of NCC is not associated with persistent and marked changes in renal hemodynamics. Like the loop diuretics, thiazides act from the luminal side and gain access to it by tubular secretion. Loss of function mutations in the NCC gene have been identified as the cause of **Gitelman's syndrome**, an electrolyte disorder characterized by hypokalemic metabolic alkalosis, magnesium wasting, and hypercalcemia.

Collecting Duct Diuretics

Na uptake through ENaC in cortical and probably also in inner medullary collecting ducts is inhibited by the so-called K-sparing diuretics (amiloride and triamterene). The natriuretic effect of this class of diuretics is small, commensurate with the small fraction of filtered Na normally absorbed along the collecting duct. Clinically perhaps more important than their natriuretic effect is the inhibition of K secretion that results from a reduced Na flux through ENaC. Inhibition of electrogenic Na absorption causes a hyperpolarization of the apical membrane and therefore a reduction in the electrochemical driving force for K secretion. The result is a reduction in K secretion and excretion since the tubular site of ENaC expression overlaps with that of the K channel responsible for the secretory cell to lumen K flux. Loss of function mutations in the ENaC gene

are the cause of ►**pseudohypoaldosteronism** type I, a salt losing nephropathy, and gain of function mutations cause ►**Liddle's syndrome**, a salt retaining state with severe hypertension.

A second diuretic acting along the collecting duct is spironolactone, a steroid that antagonizes the action of aldosterone. Eplerenone is a derivative of spironolactone with a shorter half-life and lower affinity for androgen and progesterone receptors and therefore reduced hormonal side effects. Spironolactone or eplerenone compete with aldosterone for the mineralocorticoid receptor and prevent the nuclear translocation of the receptor–ligand complex that is required for its genomic actions. Like amiloride, spironolactone causes a mild natriuresis and a reduction in K secretion.

Inhibition of V2 vasopressin receptors causes an increase in urine volume primarily by reducing the reabsorption of water along the collecting duct, an aquaretic effect that is fundamentally different from the natriuretic actions discussed so far. Nevertheless, some of the conditions calling for the use of natriuretic intervention are identical to those in which the administration of a new class of orally active nonpeptide V2 antagonists may be useful (tolvaptan, lixivaptan, and others).

Vasodilator Diuretics

Because renal vasodilatation and hyperfiltration are often associated with a natriuretic response, a number of activators or inhibitors of endogenous vasoactive systems can cause increased NaCl excretion, and some of these may be developed into compounds of clinical interest in special situations. Such agents include natriuretic peptides most notably B-type natriuretic peptide (nesiritide), neutral endopeptidase (NEP) inhibitors (thiorphan, phosphoramidon), mixed NEP and ACE inhibitors (omapatrilat), guanylin and uroguanylin, kinins, prostaglandins of the E series, adrenomedullin, relaxin, prolactin, and others.

Clinical Use CA Inhibitors

The use of CA inhibitors as diuretics is limited by their propensity to cause metabolic acidosis and hypokalemia. Their use can be indicated in patients with metabolic alkalosis and secondary hyperaldosteronism resulting for example from aggressive use of loop diuretics. Furthermore, CA inhibitors are effective drugs to produce a relatively alkaline urine for the treatment of cysteine and uric acid stones as well as for the accelerated excretion of salicylates. Perhaps the most common use of CA inhibitors is in the treatment of glaucoma.

Loop Diuretics

Loop diuretics are the drugs of choice for the treatment of edematous patients with congestive heart failure, cirrhosis of the liver, and nephrotic syndrome. Excretion of Na is helpful only to the extent that some of the

excess interstitial fluid is mobilized and shifted into the intravascular space. Aggressive diuretic therapy is only necessary in pulmonary ►**edema** resulting from left ventricular heart failure. In all edematous conditions, the diuretic treatment addresses a symptom, not the cause of the disease, and it is therefore not the therapeutic mainstay. Other clinical situations where loop diuretics are indicated include hypercalcemia, hyperkalemia, and hypermagnesemia. Loop diuretics have also been found to be advantageous in the treatment of asthma.

Adverse side effects of loop diuretics are mainly the consequence of the altered absorptive function along the loop of Henle. The most common side effect of diuretic treatment is the loss of potassium that may result in ►**hypokalemia**. K loss is a consequence of (i) reduced K reabsorption along the TAL due to the direct inhibition of NKCC2, (ii) increased K secretion caused by the combined effects of increased tubular fluid flow along the K secreting cortical collecting duct, and (iii) increased plasma aldosterone resulting from diuretic-induced hypovolemia. Furthermore, loop diuretics can cause metabolic alkalosis and the urinary loss of calcium and magnesium. NKCC1, a more widely expressed isoform of the cotransporter, is found in the inner ear, and it is involved in maintaining the unusual ionic composition of the endolymph. By interfering with this process loop diuretics at high doses, especially ethacrynic acid, can cause hearing impairment that is typically reversible, but can also lead to permanent deafness.

Thiazide Diuretics

Interference with Na absorption in the distal convoluted tubule by thiazide-like diuretics is effective in the therapy of arterial hypertension. On average, about half of patients with essential hypertension respond to thiazide monotherapy with a blood pressure reduction of more than 10%. While the initial reduction in blood pressure appears to be a consequence of a reduced plasma volume, venous return, and cardiac output, the prolonged effect of thiazides to reduce blood pressure is related to a reduction in total peripheral resistance. The cause for this long-term adjustment is multifactorial. The hypotensive effect of thiazides is enhanced by a low NaCl diet and by combination with other blood pressure lowering drugs. Often in combination with loop diuretics thiazides are also used in the treatment of edematous diseases. Like loop diuretics thiazides can cause K loss and hypokalemia, hyponatremia, and urinary magnesium wasting while they usually reduce the excretion of calcium. This characteristic feature to stimulate Ca reabsorption makes thiazides a useful drug in the prevention of calcium stone formation in ►**idiopathic hypercalciuria**. Thiazides have also been described to diminish the occurrence of bone loss, hip fractures, and osteoporosis.

K-Sparing Drugs

Amiloride, triamterene, or spironolactone are typically not used to augment the excretion of Na, but to counteract the kaliuretic effect of loop diuretics and thiazides as an alternative to dietary K supplements. Thus, K-sparing drugs often accompany treatment with loop diuretics and are useful in other K-wasting states such as primary hyperaldosteronism or Bartter's syndrome. Another beneficial effect of amiloride is a stimulation of calcium and magnesium reabsorption along distal tubules and collecting ducts, an action that counteracts the Ca and Mg wasting of loop diuretics and the Mg wasting of thiazides. Spironolactone requires functional adrenal glands and is most effective in patients with elevated plasma aldosterone levels such as those with cirrhosis of the liver and ascites. Interestingly, spironolactone or eplerenone have been found to exert beneficial cardioprotective effects as an adjuvant therapy in the treatment of heart failure.

Vasopressin Antagonists

The use of V2 antagonists is promising in the treatment of the hyponatremia that usually accompanies congestive heart failure and cirrhosis, two edematous conditions in which the use of diuretics is indicated. In addition, V2 antagonists may be beneficial in the treatment of polycystic kidney disease.

- ▶ Vasopressin/Oxytocin
- ▶ Epithelial Na⁺ Channel
- ▶ Antihypertensive Drugs

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DNA Fragmentation

DNA fragmentation occurs in two stages. Firstly, endonucleolytic activity cleaves the DNA into high molecular weight fragments leading to the morphological picture of chromatin condensation characteristic

of apoptosis. The second stage involves a calcium-magnesium endonuclease which catalyses the further fragmentation of the DNA into oligonucleosomal fragments, which give rise to the pattern of DNA base pairing which shows up as the classical DNA ladder pattern by electrophoresis. In several populations of lymphocytes, namely B lymphocyte populations in the germinal centre of lymph nodes, immature thymocytes, and cytotoxic T lymphocytes, it has been noted that only the first stage of DNA fragmentation is occurring.

- ▶ Apoptosis

DNA Methylation

In higher eukaryotes, most of the chromosomal DNA carries 5-methyl-cytidine residues located in CpG sequence motifs. There is a close correlation between transcriptional inactivation and methylation. On the other hand, considerable evidence shows that regions of DNA that are actively engaged in transcription lack 5-methyl-cytidine nucleotides in CpG motifs. Hence DNA methylation is a means how cells regulate gene expression. DNA methylation which is catalyzed by DNA methyltransferases is the best characterized epigenetic mechanism.

- ▶ Epigenetics
- ▶ Cell cycle Control

DNA Replication

DNA synthesis during S phase of the cell cycle resulting in a doubling of the genomic DNA. Replication can be subdivided into three distinct phases: initiation, elongation, and termination.

- ▶ Cell Cycle Control

DNA Response Elements

DNA response elements are generally found a short distance upstream of promoters in selected genes. They are specific for selective transcription factors and

thereby control the expression of genes regulated by these factors. The response elements often consist of small sequence repeats (or inverted repeats) separated by a variable number of DNA base pairs.

- Transcriptional Regulation
- Glucocorticoids

DNA Vaccination and Genetic Vaccination

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Definition

The administration of purified antigen-encoding plasmid DNA to a human being or laboratory animal with the aim of inducing cellular and/or humoral immune responses against the antigen is called genetic vaccination or DNA vaccination. Although less common, plasmid DNA can be replaced by a protein-encoding mRNA with the benefit of meeting high safety standards as this strategy completely eliminates the risk of integration of the plasmid DNA into the host chromosomes. In a more extended view, genetic vaccination also includes the use of recombinant viral vector systems in order to improve the delivery of the genetic information (for a more comprehensive overview see [1]).

Basic Mechanism

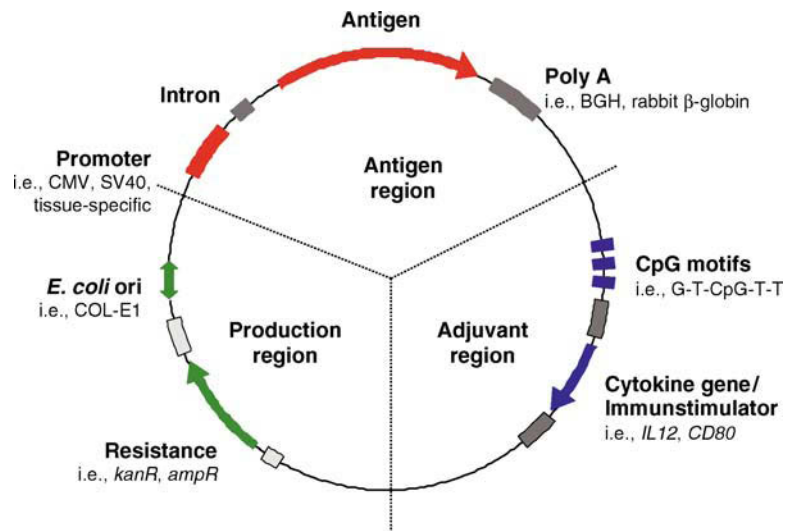
In contrast to conventional vaccines, the antigen against which an immune response is to be evoked is not delivered as a protein but as an antigen-encoding gene embedded in a eukaryotic expression vector (► [eukaryotic expression cassette](#), see [Figure 1](#)). Compared with other vaccines (e.g., purified recombinant proteins, attenuated or inactivated live virus), DNA vaccines are of great simplicity and therefore can be produced easily and are cost-effective under standardized conditions even for an intentional use in human clinical trials. Several different application routes for the DNA currently exist. The DNA is administered either via simple injection of a buffered solution into the muscle or intradermally or with the aid of delivery vehicles (gold particles in combination with a ► [gene gun](#), needle-injection devices, liposome-formulations). The route of application strongly affects both strength

and nature of the induced immune response (humoral vs. cellular immune response). A major advantage of DNA vaccines is that they have the potency to induce cytotoxic T-cell responses against the viral antigen that are otherwise difficult to achieve using conventional vaccines, with the exception of live attenuated vaccines. DNA vaccines have been successfully used as a powerful tool for the vaccination of laboratory animals against various different antigens. Because of its success under laboratory conditions, DNA vaccines meanwhile have been analyzed in numerous studies involving human subjects under clinical settings. However, at this time there is yet no DNA vaccine licensed for use in humans mainly because of difficulties of translating the promising findings from studies with small laboratory animals to human or nonhuman primates. Although DNA vaccination was proven to be safe and well-tolerated by the human study subjects in a number of phase I clinical trials, its efficacy in eliciting an antigen-specific immune-response was in most instances rather disappointing. Therefore, research is currently focusing on methods to improve the DNA-vaccine efficacy in humans. It should be noted that the application of nucleic acids to patients may also be performed for reasons other than vaccination: delivery of naked DNA/RNA could be used, e.g., for gene therapy or gene silencing.

Principles of DNA Vaccines, Mechanisms of Action

The field of DNA vaccination started when eukaryotic expression vectors were injected into the muscle of laboratory animals [2]. The authors observed protein expression for more than 2 months after injection and noted that no special delivery system was required to obtain this expression. Subsequently, it was demonstrated that antibodies can be induced simply by injecting plasmid DNA into the muscle of mice [3]. Subsequent studies found that the injection of expression plasmids also leads to the induction of a cytotoxic T-cell response. After injection, the DNA enters cells of the vaccinated host and the encoded gene becomes expressed. This eventually leads to the induction of a cellular cytotoxic T-cell, T-helper, and/or humoral (antibody) immune response.

Although a humoral immune response is the primary protection against most viral and some bacterial diseases, protective defense against other pathogens such as HIV, *Plasmodium* and *Mycobacterium tuberculosis* requires a cytotoxic response mediated by CD8⁺ T-cells (CTL response). Since the introduction of the vaccination concept by Jenner almost 200 years ago, only few vaccines have been developed that are able to induce a CTL response. These vaccines are usually attenuated live vaccines that are accompanied by certain risks and are not readily available for most pathogens. The immense appeal of DNA vaccines can be attributed to a considerable part to the fact that they are able to induce



DNA Vaccination and Genetic Vaccination. Figure 1 Plasmid vector for DNA vaccination. Three functionally distinct regions can be distinguished in a typical vaccination vector: (i) *Antigen region* contains the gene encoding the antigen of interest under the control of either a strong viral, a tissue type-specific cellular or even a synthetic promoter. Nuclear export and stability of mRNA require the presence of polyadenylation (poly A) sequences. In many instances, expression can be enhanced by insertion of a small intron upstream of the gene of interest. (ii) *Adjuvant region* CpG DNA motifs (cytosine-phosphate-guanosine as part of the sequence GTCpGTT for humans or GACpGTT for mice) of prokaryotic origin stimulate cytokine production in vivo [4]. Optionally, genes for cytokines or co-stimulatory molecules (e.g., IL-4, IL-12, IL-18, GM-CSF, B7.1), can be co-delivered with the antigen gene to improve the immune response, these elements could also be encoded by a co-administered second plasmid. (iii) *Production unit* contains elements for the production of the plasmid in bacteria. This unit includes an origin of replication, antibiotic resistance gene or other selectable marker. Note that the *ampicillin* resistance gene cannot be used in clinical trials involving human subjects. Instead, there are now nonantibiotic based selectable markers for plasmid maintenance in *Escherichia coli* available, which function by repressor titration strategies.

long-lasting CTL responses, next to the ease of their production. Additionally, since antigen expression occurs in an authentic intracellular environment, antigens have a high probability to fold into their native conformation as well as acquire relevant posttranslational modifications and thus have a great potential to induce effective antibody responses. Other advantages of DNA vaccines include (i) their low production costs as generic manufacturing procedures can be applied, (ii) clinically proven safety as compared with live attenuated vaccines, and (iii) high physical stability not requiring intact cold chains. Standardized production protocols, which are independent on the encoded antigen, would allow shorter response times to provide protection against, e.g., Influenza pandemics; however, this head start is consumed by the requirement for three successive immunizations to achieve protective antibody titers using a DNA-based flu vaccine.

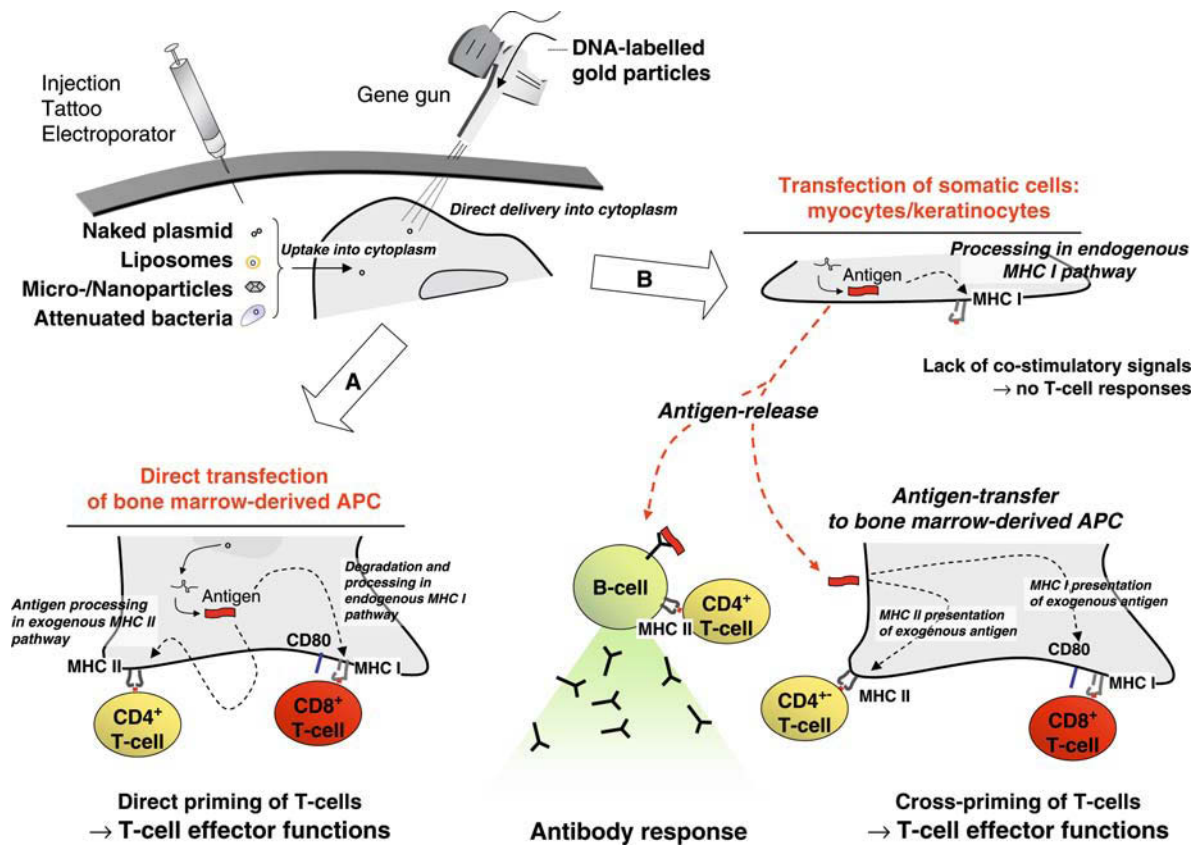
Pharmacological Relevance

There are various protocols of administering eukaryotic expression vectors aiming to deliver (i.e. transfect) the DNA into the cytoplasm of the host cells (see Figure 2). The DNA is subsequently imported into the nucleus of the transfected cells allowing expression of the

encoded antigen. To induce antigen-specific, CD8⁺ **▶cytotoxic T-cells**, the antigen has to be presented by professional **▶antigen-presenting cells** (APCs). It is still under discussion whether DNA vaccination requires direct **▶transfection** of APCs (see Fig. 2A) or whether delivery of the DNA into nonimmune cells (e.g., muscle cells; Fig. 2B) is sufficient. In the latter scenario, there are a number of pathways that allow transfer of the antigen from nonimmune cells to APCs, a process that is called cross-priming.

The resulting immune response shows qualitative differences based on the method used for DNA vaccination. Injection of naked plasmid DNA predominantly leads to the induction of a strong cytotoxic T-cell response. In contrast, if the DNA is delivered via gold-particle bombardment using a gene gun, the immune response is biased towards a humoral response. The reason for these differences is based on the fact that gold particle bombardment is thought to deliver the DNA directly into the cytoplasm of the host cells. This bypasses the interaction of the bacterially derived vector DNA with specialized **▶“toll-like”-receptors** (TLR) present on the surface of many cells.

TLRs are the basis for the fact that plasmid DNA of prokaryotic origin, as is used in all DNA vaccination



DNA Vaccination and Genetic Vaccination. Figure 2 Mechanisms of DNA vaccination. Via needle injection or with the aid of specific devices (gene gun, needle injector, tattoo device), the expression construct is delivered indirectly or directly into the cytoplasm and subsequently the nucleus of cells *in vivo*. Alternatively, noninvasive methods, such as aerosols and viral vectors are employed in order to improve DNA delivery. Following uptake of the DNA, the antigen-encoding gene becomes expressed by the transfected cell. Two major hypotheses have been proposed for the induction of a cellular immune response. (A) In this scenario the DNA is delivered directly to specialized immune cells, the professional antigen-presenting cells (APCs). These cells have the ability to process the expressed antigen and present it to both CD8⁺ T-cells of the immune system in the context of the major histocompatibility complex class I (MHC I) and CD4⁺ T-cells in context of MHC II. The T-cells become efficiently activated since the professional APCs provide the required co-stimulatory signals (i.e. CD80 or CD86 of APCs binding to the CD28 receptor on T-cells). (B) Alternative mechanism for the induction of a cellular response (called cross-priming). In this setting, the antigen becomes expressed and presented by somatic, nonimmune cells. Since these cells lack the co-stimulatory signals, this presentation does not lead to the activation of CD8⁺ effector cells. It has been shown, however, that the expressed antigen can be released from the somatic cells and thereby enables the activation of both arms of the adaptive immune system (i) the released antigen can be recognized by circulating B-cells, which initiate the production of antigen-specific antibodies. This humoral immune response is further stimulated by CD4⁺ T-helper cells that become activated by APCs presenting the antigen through the MHC class II pathway. (ii) CD8⁺ cytotoxic T-cell responses can be induced by a specialized subset of bone marrow derived APCs, which allow MHC-I presentation of exogenous antigens in the context of co-stimulatory molecules – a process termed cross-presentation or cross-priming. In addition to the two scenarios above, it has been found that there is an alternative co-stimulatory molecule (BB-1) expressed by myocytes in muscles upon induction of inflammation. However, there is strong evidence that bone marrow-derived APCs are essential for the induction of CTL responses during the course of DNA vaccination.

protocols, not only exhibits an inherent adjuvant effect on the stimulation of immune responses against the vector-encoded antigen but also against other antigens if they are co-administered with the DNA. The reason of this adjuvant effect lies in the presence of so-called CpG (5'-Cytosine-phosphate-Guanosine-3') motifs in

the vector backbones [5]. These motifs are, in comparison with prokaryotes, underrepresented and usually methylated in mammalian genomes. Contact with a significant amount of unmethylated CpG motifs during the course of bacterial infection or following vaccination with plasmid DNA of prokaryotic origin, leads to the activation

of immune cells expressing the TLR9. Consequently, these cells secrete immune stimulatory ►cytokines that can boost the induced immune response.

Potential Risks

In general, currently available preclinical and clinical data indicate that DNA vaccination is safe and well-tolerated [5]. Available data show that there is no indication of integration of the injected DNA into the host genome and therefore the risk of causing cancer by insertional mutagenesis is generally thought to be negligible. Additionally, in none of the clinical or preclinical studies there have been indications for the induction of anti-DNA antibodies believed to be key factors in certain autoimmune diseases. Anyway, even without DNA vaccination, humans are frequently exposed to bacterial DNA without evidence for adverse consequences. However, several safety concerns remain unanswered at present and have to be evaluated in the future. Immuno-regulatory cytokines induced by the CpG motifs present on the DNA vaccine may interfere with the host immune response either leading to autoimmune diseases or inducing tolerance to human pathogens that are present at the time of vaccination. Additional concerns are raised when, in addition to the antigen of interest, genes encoding immuno-stimulatory proteins such as cytokines are co-administered. It has been demonstrated that plasmid DNA can persist for weeks or even years post-vaccination. The consequences of such long-term exposure to foreign antigens, especially in respect to tolerance and long-lasting memory responses, are still not clear. These consequences of DNA vaccination have to be evaluated carefully, especially since most vaccine applications that are currently in evaluation target newborns or young children.

Clinical Use

A large and rapidly growing number of clinical trials (phase I and phase II) evaluating the potential of DNA vaccines to treat and prevent a variety of human diseases are currently being performed (►<http://clinicaltrials.gov>); however, there is yet no licensed DNA vaccine product available for use in humans. The clinical trials include the treatment of various types of cancers (e.g., melanoma, breast, renal, lymphoma, prostate, and pancreas) and also the prevention and therapy of infectious diseases (e.g., HIV/AIDS, malaria, Hepatitis B virus, Influenza virus, and Dengue virus). So far, no principally adverse effects have been reported from these trials. The main challenge for the development of DNA vaccines for use in humans is to improve the rather weak potency. DNA vaccines are already commercially available for veterinary medicine for prevention of West Nile Virus infections in horses and Infectious Hematopoietic Necrosis Virus in Salmon.

Routes of Administration/Vehicles for Administration/Formulation

Since, upon injection of naked plasmid DNA, the vast majority of DNA becomes readily degraded by cellular nucleases, efforts have been undertaken to increase the number of DNA molecules reaching the nucleus of cells. DNA molecules can be introduced into the cytoplasm of cells by the use of gold particle bombardment via a gene gun. This reduces the amount of DNA required for induction of an immune response to 1/10th compared with the injection of plasmid DNA and pressure-powered delivery devices licensed for use in humans are available (e.g., PowderMed™). In addition, a number of routes for DNA injection (intramuscular, intradermal, intraperitoneal, epidermal, intranasal, and intravenous) have been investigated. In most protocols, a dose of 1–100 µg of DNA per administration is used for small laboratory animals and 100–4000 µg for humans. However, only a minute fraction of the DNA molecules actually arrive in the nucleus and lead to the production of protein in the pico-to-nanogram range over a time period of weeks to several months. Recently, several alternative methods have been applied for DNA delivery. First, the use of a tattooing device for genetic vaccination of laboratory animals was shown to strongly improve the vaccine efficacy. Second, a growing number of trials involving animal and human subjects have used a method called ‘*in vivo* electroporation’ in order to deliver the DNA. Here, a brief electronic pulse is applied to the site of DNA injection. This method proved to be highly efficient in DNA delivery, however, it is accompanied by a (short-lived) moderate-to-serious discomfort for the vaccinee, acceptable only under therapeutic settings. In addition, it has been reported that electroporation leads to an increased frequency of integration of plasmids into the host genome. Finally, it has been demonstrated that oral delivery of a DNA vaccine can be significantly improved by the use of nasal sprays in combination with viral vector systems that are able to target these tissues. In addition to the incorporation of immune-modulatory genes as ‘genetic adjuvants’, a number of other formulations have been investigated in order to improve DNA vaccination. DNA molecules can be delivered by liposome formulation using commercial reagents such as Lipofectamine, Transfectam, and Bupivacaine, which allow a more efficient cellular uptake of the DNA and possibly also provide some degree of an adjuvant effect. For protection against degradation and to allow deposit effects, DNA vaccines can be microencapsulated by the use of polymers such as Poly-lactide-co-glycolide or chitosan. QS21, a saponin that is derived from the bark of the *Quillaja saponaria* tree was shown to function as adjuvant in DNA vaccination.

- Immune Defense
- T Cell Receptors

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blood–brain barrier (e.g. carbidopa), reduce the toxicity and peripheral side-effects of L-DOPA during treatment of Parkinson's disease.

- Dopaminergic System
- Anti-Parkinson Drugs
- α -Adrenergic System

Docking Protein

- Adaptor Proteins

Domain

A domain is a part of a larger protein which has distinctive structural or functional properties.

L-DOPA/Levodopa

The immediate metabolic precursor to dopamine, L-DOPA (L-dihydroxyphenylalanine) is converted to the active neurotransmitter dopamine by the action of the enzyme aromatic amine acid decarboxylase (AADC). L-DOPA (INN name Levodopa) is the main drug used to treat Parkinson's disease.

- Dopamine System
- Catechol-O-Methyltransferase and its Inhibitors
- Anti-Parkinson Drugs

Dopa Decarboxylase

Dopa decarboxylase is an enzyme catalyzing the synthesis of dopamine from L-DOPA or of serotonin (= 5-hydroxytryptamine) from L-tryptophan. Inhibitors of this enzyme, which do not pass through the

Dopamine System

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Synonyms

Dopamine: 3,4-dihydroxyphenylethylamine

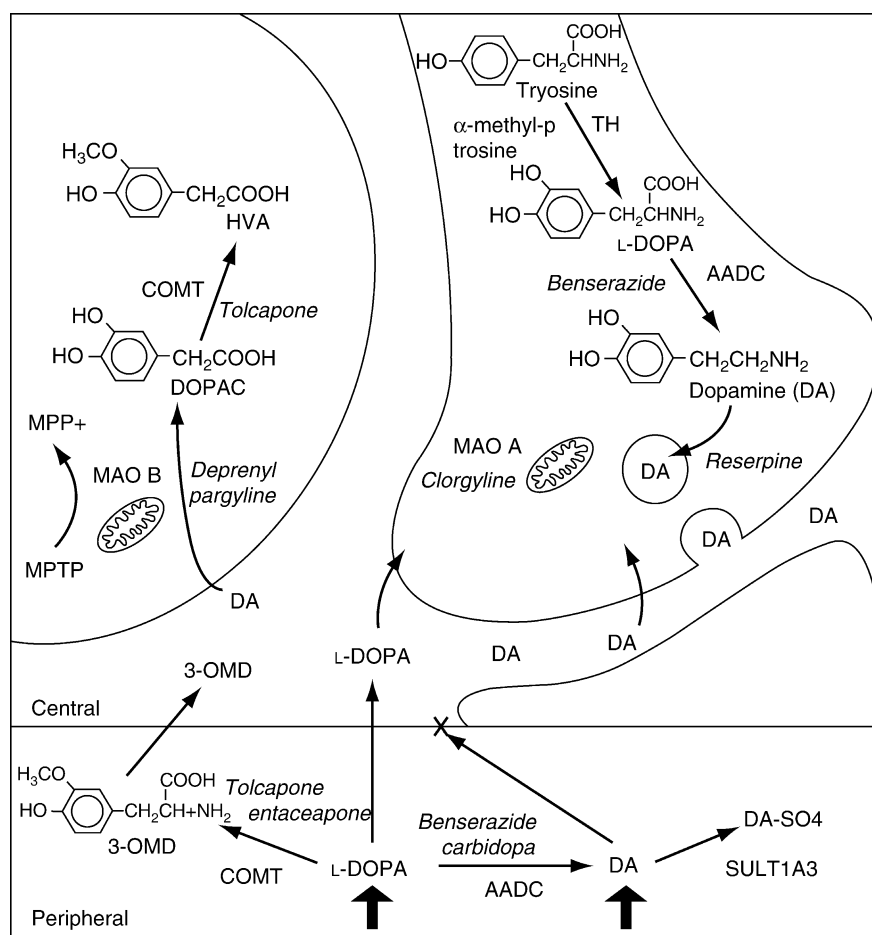
Definition

The dopamine system constitutes the cellular and biochemical network that is involved in the synthesis, release, and response to dopamine. In general, this involves cells that express significant levels of ► **tyrosine hydroxylase** (TH) and limited amounts of dopamine β -hydroxylase [1]. Dopamine-responsive cells express receptors specifically activated by this neurotransmitter, which are known as dopamine D1, D2, D3, D4, and D5 receptors [2, 3].

Characteristics and Basic Mechanisms Anatomy and Function

Dopamine is one of the main neurotransmitters in the central nervous system (CNS), but has also been reported to play a role in the periphery. In the CNS, dopamine-synthesizing neurons have been found in a number of discrete cell groups in the mid- and forebrain, designated as A8-A10 (midbrain areas), A11-A15 (hypothalamic areas), A16 (olfactory bulb), and A17 (retina). The main dopaminergic neurons are found in the substantia nigra pars compacta and in the ventral tegmental area, which are also designated as the A9 and A10 cell groups and constitute the nigrostriatal and mesocortical/mesolimbic systems, respectively (Fig. 1).

The nigrostriatal system is predominantly involved in motor control, which is particularly evident in ► **Parkinson's disease** (PD), where a progressive loss of these neurons results in loss of motor function. In the early stages of the disorder, the motor impairment can be reversed by the administration of the dopamine precursor ► **L-DOPA** (L-3,4-dihydroxyphenylalanine), which bypasses the need for TH in dopamine



Dopamine System. Figure 1 Dopamine synthesis and metabolism in humans. The enzyme tyrosine hydroxylase (TH) catalyzes the formation of L-DOPA from tyrosine in presynaptic catecholaminergic neurons. L-DOPA (L-3,4-dihydroxyphenylalanine) is converted to dopamine by AADC (aromatic amino acid decarboxylase) and taken up by vesicles through the vesicular monoamine transporter (VMAT). After the release of vesicular dopamine into the synapse, signaling is terminated by the reuptake of dopamine into the presynaptic terminal by the dopamine transporter (DAT). Dopamine that leaves the synapse is primarily inactivated by the sequential action of monoamine oxidase (MAO) B and catecholamine-O-methyl transferase (COMT), which catalyzes the formation of DOPAC (3,4-dihydroxyphenylacetic acid) and HVA (homovanillic acid), respectively. MAO B also catalyzes the formation of the neurotoxin MPP⁺ from MPTP. Although MAO A can also oxidize dopamine, it does not appear to carry out this role in humans. In the periphery, dietary dopamine is primarily inactivated by the action of the phenyl sulfotransferase SULT1A3. Dopamine sulfate (DA-SO₄) is the predominant form of dopamine in human plasma. In Parkinson's disease patients, L-DOPA treatment results in a large increase in peripheral dopamine metabolites such as DA-SO₄ and 3-OMD (3-O-methyldopa). Drugs such as carbidopa, benserazide, tolcapone, and entacapone are used clinically to increase the brain availability of L-DOPA. The selective MAO B inhibitor deprenyl is used to delay the progression of PD symptoms and enhance the effectiveness of L-DOPA therapy.

synthesis [1]. The mesocortical and mesolimbic systems play important roles in reward, emotion, and cognition. This is exemplified by the addictive properties of dopamine stimulants, such as cocaine and amphetamine, as well as the therapeutic properties of drugs that block the D2 class of dopamine receptors, which control the psychotic symptoms of **schizophrenia** (see below). Dopaminergic hypothalamic neurons in the arcuate nucleus (A12) and A14 cell groups form the

tuberoinfundibular system. These neurons project to the median eminence and control the release of prolactin through the hypophyseal portal system. More dorsally located dopamine neurons in the hypothalamus (A11, A13) project to autonomic areas of the lower brain stem and preganglionic sympathetic neurons of the spinal cord. Dopamine in the retina has been found in the amacrine and interplexiform cells and is involved in light and dark adaptation.

In the periphery, potential physiological roles of dopamine are less well established. The main roles for peripheral dopamine appear in the control of blood pressure. Dopamine affects vasodilation by sympathetic and renal mechanisms involving hemodynamic and direct effects on the nephron as well as effects on renin secretion. Furthermore, dopamine can modulate the secretion of aldosterone from the adrenals. Peripheral dopamine is also postulated to function in the gastrointestinal system. In this respect, it is important to note the antiemetic effects of the peripheral D2 class blockers, such as domperidone. These drugs presumably act via interaction with receptors in the chemoreceptor trigger zone. The origin of peripheral dopamine is not entirely resolved. Most of the dopamine in the circulation is in an inactivated form conjugated to sulphate or glucuronide. Origins of peripheral dopamine are thought to be from the sympathetic neurons, mesenteric organs, and adrenals.

Genetic disruption of dopamine synthesis in mice lacking TH shows that dopamine is not essential for development. However, dopamine deficient mice do not survive long after weaning unless treated with L-DOPA. These mice display severe aphagia and adipsia and loss of motor function. While these mice have a major reduction in dopamine levels some residual dopamine can be detected that is generated through the action of tyrosinases.

Dopamine Synthesis and Metabolism

The rate-limiting step in dopamine biosynthesis is the conversion of L-tyrosine to L-DOPA by the enzyme tyrosine hydroxylase (TH) [1]. TH is expressed in catecholaminergic neurons in the brain, sympathetic ganglia, and adrenal medulla. The monooxygenase activity of TH is dependent on the cofactor tetrahydropterin (BH4), and nigrostriatal neurons are particularly sensitive to BH4 levels. Genetic mutation in GTP cyclohydrolase I, which regulates production of BH4, is the cause of HPD (hereditary progressive dystonia). In catecholaminergic neurons, the enzyme aromatic amino acid decarboxylase (AADC) (also known as dopamine decarboxylase) catalyzes the formation of dopamine from L-DOPA. Dopamine is taken up into vesicles by the vesicular monoamine transporter (VMAT). In humans, peripheral dopamine formed from L-DOPA or absorbed intestinally is rapidly converted to inactive dopamine sulfate by the phenol sulfotransferase SULT1A3. In some species such as rats, the major form of dopamine in plasma is glucuronide conjugated [4].

The principal mechanism for terminating dopamine signaling is reuptake by the presynaptic neuron via the dopamine transporter (DAT). Dopamine that is not taken up is metabolized by the enzymes ►monoamine oxidase (MAO) and ►catechol-*O*-methyl transferase

(COMT) [5, 6]. MAO is an FAD (flavin adenine dinucleotide)-dependent enzyme with two distinct forms, MAO A and MAO B. Both occur on the outer mitochondrial membrane, but they have distinct localization and substrate specificity. MAOs are involved in inactivating neuroactive amines in the brain and periphery as well as detoxification of xenobiotics. Low levels of MAO A have been observed in dopaminergic neurons. However, dopamine oxidation in humans occurs primarily through MAO B, which is located in serotonergic neurons and glial cells. The distinction between the sites of dopamine biosynthesis and MAO B expression suggests that its role is to prevent dopamine from affecting nondopaminergic neurons. MAO A is selectively inhibited by clorgyline and moclobemide while MAO B is blocked by the reversible inhibitor deprenyl. Deprenyl also protects nigrostriatal neurons from the PD-like effect of the drug MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). MAO B activity is required to catalyze the formation of neurotoxic MPP⁺ (1-methyl-4-phenylpyridinium ion), which is selectively taken up by nigrostriatal neurons resulting in Parkinsonism. MAO B activity increases with aging, and deprenyl has been shown to delay the progression of PD, although its precise mechanism of action is currently unknown.

MAO converts dopamine to DOPAC (3,4-dihydroxyphenylacetic acid), which can be further metabolized by COMT to form homovanillic acid (HVA). HVA is the main product of dopamine metabolism and the principal dopamine metabolite in urine. Increased neuronal dopaminergic activity is associated with increases in plasma concentrations of DOPAC and HVA. COMT preferentially methylates dopamine at the 3'-hydroxyl position and utilizes *S*-adenosyl-L-methionine as a methyl group donor. COMT is expressed widely in the periphery and in glial cells. In PD, COMT has been targeted since it can convert L-DOPA to inactive 3-OMD (3-*O*-methyl-dopa). In the presence of an AADC inhibitor such as carbidopa, 3-OMD is the major metabolite of L-DOPA treatment.

Dopamine Receptors

To date, five different dopamine receptors have been identified in and cloned from mammalian organisms [2, 3]. These are classified as dopamine D1 (D1a), D5 (D1b), D2, D3, and D4 receptors. The genes for the D1 and D5 receptors have two exons and are intronless in the coding sequence, while the D2, D3, and D4 receptors have eight, six, and four exons, respectively. Through alternative splicing of the sixth exon (fifth in coding sequence), the human D2 receptor gene can generate two forms of the receptor, which are called D2long (D2L, D2(443)) and D2short (D2S, D2(414)). The dopamine receptors belong to the superfamily of ►G protein-coupled receptors (GPCR). Most structural

data on dopamine receptors have been derived from mutagenesis studies, by analogy with the structure of other catecholamine receptors and from structural information on the prototypic class I GPCR, rhodopsin. Critical amino acid residues for dopamine binding and receptor activation are an aspartate in transmembrane domain three, which serves as counterion for the primary amine of dopamine, and the two serine residues in transmembrane domain five, which are involved in hydrogen bonding with the hydroxyl groups of the catechol ring. Dopamine has access to these residues through a hydrophilic pocket that is presumably formed through the counter-clockwise orientation of the transmembrane domains. To date there are no crystallographic data on the dopamine receptors. As with other GPCR, it has been shown that dopamine receptors, particularly D2 receptors, can form homo- and hetero-oligomeric structures. Whether the receptor exists as monomeric or oligomeric form in vivo or is regulated in a functionally dynamic fashion is still unknown.

Functionally, the D1-like receptors (D1, D5) are coupled to the G protein G_{α_s} and thus can stimulate adenylyl cyclase. The D2-like receptors (D2, D3, and D4) couple to pertussis toxin sensitive G proteins ($G_{\alpha_{i/o}}$), and consequently inhibit adenylyl cyclase activity. While the D1-like receptors almost exclusively signal through G_{α_s} -mediated activation of adenylyl cyclase, the D2-like receptors have been reported to modulate the activity of a plethora of signaling molecules and pathways. Many of these actions are mediated through the $G\beta\gamma$ subunit. Some of these molecules and pathways include the calcium channels, potassium channels, sodium-hydrogen exchanger, arachidonic acid release, and mitogen-activated protein kinase pathways.

Direct interactions of dopamine receptors with signaling, regulatory, and structural molecules have also been reported. This includes the interaction of D1 receptors with calcyon, D5 receptors with GABAA channel subunits, D2 receptors with spinophilin and actin-binding protein (ABP-280), and D2-like receptors with SH3 domain-containing proteins. Dopamine receptors may be substrates for GPCR kinases (GRK) and arrestins, regulating the activity of the receptors through GRK-mediated phosphorylation and subsequent increased affinity for arrestins. Repeated and/or extended activation of many GPCRs, including dopamine receptors, results in reduced responsiveness. This desensitization process is mediated by the association of arrestin to GPCR. Regulation of dopamine receptors by GRK and arrestins is shown for D1 and D2 receptors, but not yet extensively studied. Most of the functional activities have been examined in vitro and in heterologous expression systems using recombinant receptors. The particular use of any signaling

pathway/molecule in vivo is largely dependent on the cellular phenotype of these cells.

Dopamine receptors are widely expressed in the brain. The main areas of D1 receptor expression are the caudate nucleus, putamen, and accumbens, with lower levels in the neocortex, hippocampus, and amygdala. The D5 receptor is expressed at very low levels in the brain, predominantly in limbic areas. The D2 receptor is also expressed highly in the caudate nucleus, putamen, accumbens, and islands of Calleja. D2 receptor levels in the neocortex, amygdala, and hippocampus are in the same order of magnitude as D1 receptor levels. Expression of the D2 receptor in the dopamine neurons of the substantia nigra and ventral tegmental indicate that it serves as a so-called dopamine autoreceptor. Unlike the D1 receptor, the D2 receptor is expressed in the pituitary gland, most notably in the mammotrophic cells of the anterior pituitary. It has been reported that the alternatively spliced short form (D2S) is preferentially expressed in the dopaminergic neurons of the substantia nigra, while the long isoform (D2L) is expressed in postsynaptic areas. The D3 receptor is expressed at intermediate levels in brain, most notably in the shell region of the accumbens and Islands of Calleja and at lower levels in dopaminergic neurons. The D4 receptors are widely expressed through the brain at intermediate to low levels. D4 receptor expression has been observed in the retina, neocortex, hippocampus, and amygdala.

Clearly, the highest levels of expression of D1 and D2 receptors are seen in the striatum. The levels of expression are at least one order of magnitude higher than in any other brain region or for any of the other receptors. The D1 and D2 receptors are expressed in the medium spiny neurons of this region. The majority of these receptors are not colocalized in this region and form, respectively, the so-called direct and indirect pathways to the output neurons of the basal ganglia. In cortical areas, dopamine receptors are found in the pyramidal neurons and interneurons. D2 receptors have also been found in cortical astroglia. The dopamine receptors are found in the terminal fields of dopaminergic projections and a significant proportion of dopamine-mediated modulation is via "volume-control."

In the periphery, dopamine receptor levels are generally lower than those observed in brain, particularly in comparison to striatal dopamine receptor levels. Due to these low levels, knowledge of receptor distribution in the periphery is not yet comprehensive. Nevertheless, D1-like receptors have been reported in the parathyroid gland and in the tubular cells of the kidney. D2-like dopamine receptors have also been observed in the kidney. In addition, dopamine D2 and D4 receptors have been found in the adrenal cortex, where they modulate aldosterone secretion. The

dopamine D4 receptor has been detected at relatively high levels in the cardiac atrium. Furthermore, D1- and D2-like receptors have been reported in various arterial beds (including renal, coronary, pulmonary, and cerebral arteries), the carotid body, sympathetic neurons, and the gastrointestinal tract.

The physiological roles of the different dopamine receptors in the CNS have been investigated by pharmacologic and genetic means. Pharmacologically, D1 and D5 receptors cannot be distinguished. However, D1-like receptor blockade can induce catalepsy, while mice in which the D1 receptors are genetically ablated display only minor motor control problems. However, pharmacological and genetic evidence suggest a role for D1 receptors in mediating the action of psychostimulants. D5-deficient mice have only been generated recently and no major phenotypes have yet been reported.

Drugs that block D2 receptors and D2 receptor-deficient mice have both demonstrated that these receptors have a major role in motor control, reward mechanisms, and endocrine control. Clinical pharmacological evidence indicates that the majority of antipsychotic medications mediate their effects through this receptor. While there are several D3 receptor preferring ligands, no truly selective ligands for this receptor have been identified to date. Nevertheless, pharmacological and genetic studies do not indicate a major role for this receptor in motor control, although a possible role in the response to psychostimulants is emerging. Similarly, the D4 receptor appears to play no major role in motor control. However, pharmacological evidence combined with the use of mice deficient for D4 receptors indicate that D4 receptors are involved in the response to psychostimulants and novel stimuli. Human genetic studies have provided evidence that the D3 receptor gene is a factor in the development of neuroleptic-induced tardive dyskinesia, while D4 receptors may be a genetic factor contributing to the development attention deficit hyperactivity disorder (ADHD). D1 and D3-deficient mice develop hypertension, indicating a role for dopamine receptors in blood pressure control.

Pharmacology, Drugs, and Clinical Uses

The dopamine D1-like receptor family has preferential affinity for ligands of the benzazepine class. Currently, there are no ▶[antagonists](#) or agonists that can distinguish between D1 and D5 receptors. However, the antagonist SCH23390 and agonist SKF38393 are selective for the D1-class of receptors, although no major clinical applications for D1 receptor-selective ligands have been identified.

The antipsychotic activity of ▶[neuroleptics](#) (D2-like receptor antagonists) has led to the development of many different ligands for the D2 receptor. These drugs

are used to control the psychosis that is seen in schizophrenia, as well as Huntington's disease and Alzheimer's disease. The major drug classes are the phenothiazines, butyrophenones, and benzamides, of which chlorpromazine, haloperidol, and sulpiride are examples frequently used in the clinic. Many of the so-called classic neuroleptics display an increased propensity for motor side effects (Parkinsonian-like effects), particularly when used at too high doses (over 80% D2 receptor occupancy). Atypical ▶[antipsychotics](#), like clozapine, risperidone, and olanzapine, display a limited propensity for motor side effects. These drugs have a more complex pharmacological profile that includes antagonism of 5HT, muscarinic, and α -adrenergic receptors, as well as D2 receptor block. Most of the common antipsychotics also block the D3 and D4 receptors, but the antipsychotics raclopride and sulpiride have a poor affinity for D4 receptors. Selective ligands for D3 and D4 receptors have been developed, but have no clinical use thus far. D2-like receptor blockers that cannot cross the blood-brain barrier, like domperidone, are frequently used as antiemetic/prokinetic to combat nausea and dyspepsia.

Dopamine D2-like receptor ▶[agonists](#) include quinpirole, bromocryptine, and pergolide. Selective agonists, like bromocryptine, are used in the control of prolactinomas and its associated hyperprolactinaemia. Dopamine agonists like pergolide are used in the treatment of PD. Because of their vasodilatory and renal effects, dopamine receptor agonists, including dopamine itself, have been used in the treatment of heart failure.

The dopamine precursor L-DOPA (levodopa) is commonly used in TH treatment of the symptoms of PD. L-DOPA can be absorbed in the intestinal tract and transported across the blood-brain barrier by the large neutral amino acid (LNAA) transport system, where it taken up by dopaminergic neurons and converted into dopamine by the activity of TH. In PD treatment, peripheral AADC can be blocked by carbidopa or benserazide to increase the amount of L-DOPA reaching the brain. Selective MAO B inhibitors like deprenyl (selegiline) have also been effectively used with L-DOPA therapy to reduce the metabolism of dopamine. Recently, potent and selective nitrocatechol-type COMT inhibitors such as entacapone and tolcapone have been shown to be clinically effective in improving the bioavailability of L-DOPA and potentiating its effectiveness in the treatment of PD.

- ▶[Emesis](#)
- ▶[Orexins](#)
- ▶[Neurotransmitter Transporters](#)
- ▶[Monoamine Oxidases and their Inhibitors](#)
- ▶[Catechol-O-Methyltransferases](#)
- ▶[Anti-Parkinson Drugs](#)
- ▶[Antipsychotic Drugs](#)

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Dopamine- β -hydroxylase

Dopamine hydroxylase synthesizes norepinephrine (Nor-adrenalin) from dopamine.

► α -Adrenergic System

Dopaminergic Neurotoxicity

Degeneration and death of dopamine neurons, resulting from neurotoxic agent.

- Anti-Parkinson Drugs
- Monoamine Oxidases and their Inhibitors

Dose

Amount of a drug given intravenously or orally, intermittently or continuously.

► Pharmacokinetics

Dose-response Curves

► Drug-Receptor Interaction

Double Stranded RNA

Double stranded (ds) RNA is not a constituent of a normal cells but is produced during replication of many RNA and DNA viruses either as an obligatory intermediate or as a side product. As a foreign molecule, double stranded RNA induce the secretion of interferon (IFN) from lymphocytes, neutrophils and fibroblasts.

► Interferons

Downregulation

Downregulation is the process that leads to a diminished cellular response. It can involve mechanisms at various levels.

► Tolerance and Desensitization

Drotrecogin α (Recombinant Human Activated Protein C)

► Anticoagulants

Drug Abuse

► Drug Addiction/Dependence

Drug Addiction/Dependence

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Synonyms

Drug abuse; Substance use disorders

Definition

Drug addiction is defined as a syndrome in which drug use (e.g., psychostimulants, opiates, alcohol) pervades all life activities of the user. Life becomes governed by the drug and the addicted patient can lose social compatibility (e.g., loss of partner and friends, loss of job, crime). Behavioral characteristics of this syndrome are compulsive drug use, ►**craving**, and chronic relapses that can occur even after years of abstinence.

Drug addiction is a pathological behavioral syndrome that has to be strictly separated from ►**physical dependence**. An individual can be physically dependent on a drug without being addicted to it and vice versa. Transient neuroadaptive processes underlie physical dependence and ►**tolerance** to a drug, whereas persistent changes within specific neuronal systems underlie addictive behavior.

Basic Mechanisms

Acute Drug Action/Drug Reinforcement and the Mesolimbic Dopamine System

The dopaminergic ►**mesolimbic system** is thought to serve as a final common neural pathway for mediating drug reinforcement [1]. Drugs of abuse have different primary pharmacological targets (e.g., monoamine transporters, opioid receptors, cannabinoid receptors, nicotinic receptors) but ultimately, they all activate dopamine neurons. Although drug-induced activation of mesolimbic dopamine neurons has an important function in the acquisition of behavior reinforced by drug stimuli, the subjective rewarding actions of drugs of abuse are more likely to be mediated via activation of opioidergic systems. Thus a variety of drugs of abuse including alcohol and psychostimulants stimulate endorphin release in the nucleus accumbens [2]. The endocannabinoid system is also involved in mediating brain reward functions as it has been shown that activation or blockade of the endogenous cannabinoid system modulate the rewarding effects of drugs of abuse.

Psychostimulants

Both ►**psychostimulants** D-amphetamine and cocaine elevate extracellular dopamine concentrations in the terminal region of midbrain dopamine neurons,

especially in the nucleus accumbens. Microdialysis experiments examining the dopaminergic response to cocaine in self-administering rats with high-time resolution demonstrate that responses to cocaine are regulated by changes in extracellular dopamine levels in the nucleus accumbens. Since cocaine-induced increases in extracellular dopamine concentrations are due to the blockade of presynaptic dopamine transporters (DAT), disruption of DAT should attenuate the reinforcing effects of cocaine; however, DAT knockout mice acquire self-administration of cocaine. Thus, the reinforcing actions of cocaine do not depend solely on cocaine-induced increases in synaptic dopamine. It has been found that serotonin transporters (SERT) are also involved in acute reinforcement processes of cocaine.

Opioids

In vivo microdialysis data demonstrate that acute systemic or intracerebroventricular administration of μ - or δ -opioid receptor agonists increase dopamine release in the nucleus accumbens. Opioid agonists increase extracellular dopamine levels within the nucleus accumbens by disinhibiting GABA interneurons in the ventral tegmental area. Activation of μ -opioid receptors on GABAergic interneurons hyperpolarizes these interneurons and concomitantly disinhibits dopamine cell firing. These disinhibitory actions of opioid receptor agonists are restricted to the ventral tegmental area since direct application of μ -opioid receptor agonists into the midbrain increases mesolimbic dopamine activity whereas intra-nucleus accumbens infusions do not alter extracellular dopamine levels in this structure. Opiate reward as measured by the ►**conditioned place preference** method depends on midbrain dopamine mechanisms. Microinjections of opioid receptor agonists into the ventral tegmental area, but not nucleus accumbens, induce conditioned place preference.

Alcohol

Alcohol has a complex pharmacology. Primary targets of ethanol are GABA_A, NMDA, nACh, and 5-HT₃ receptors and potassium channels as well. Alcohol increases firing of dopamine neurons in the ventral tegmental area, enhances release of dopamine from the nucleus accumbens shell region. Similar to opioid-induced stimulation of dopamine release alcohol is thought to decrease the activity of GABAergic neurons in the ventral tegmental area, which leads to a disinhibition of mesolimbic dopamine neurons. Importantly, rats will self-administer ethanol directly into the ventral tegmental cell body region of mesolimbic dopamine neurons, and both dopamine D1 and D2 antagonists administered either systemically or locally into the nucleus accumbens decrease home cage drinking and operant responding for alcohol showing that alcohol reinforcement depends on the mesolimbic system.

Molecular Mechanisms of Physical Dependence

Chronic administration of opiates and alcohol leads to physical dependence; a phenomenon, which is only weakly expressed following chronic administration of psychostimulants or other drugs of abuse. Physical dependence results from neuroadaptive intracellular changes to an altered pharmacological state. Abstinence from chronic opiate or alcohol use leads to a variety of physiological and psychological withdrawal symptoms based on these adaptations of the neuronal system.

Opioids

The opiate withdrawal symptoms in humans and experimental animals are generally the same: Elevation of temperature and blood pressure, alteration of pulse rate, restlessness, diarrhea, weight loss, anxiety and depression. Most of the physiological aspects of opiate withdrawal are based upon an over-excitability of the noradrenergic system. The locus coeruleus is the major noradrenergic nucleus in the brain and is thought to be involved in physical dependence. Chronic opiate exposure results in an up-regulation of the cAMP system. This up-regulated or “hypertrophied” cAMP system in the locus coeruleus and other brain stem nuclei is a compensatory, homeostatic response to the inhibition from chronic opiate treatment. cAMP up-regulation results in the activation of the transcription factor cAMP response binding element (►CREB). The up-regulated cAMP system has been shown to contribute to the increase in the electrical excitability of locus coeruleus neurones associated with opiate withdrawal, and transgenic mice deficient in CREB exhibit attenuated withdrawal signs compared to wild-type mice.

Alcohol

Chronic administration of ethanol leads to a variety of adaptive responses within the central nervous system that become uncovered during withdrawal. Especially, hyperexcitability and susceptibility to seizures during withdrawal are thought to be due to adaptive responses within the glutamatergic system in the hippocampus. Assuming that acute ethanol induces a reduction in glutamate release, adaptive responses such as changes in the number and affinity of synaptic glutamate receptors and glutamate transporters occur in order to keep physiological homeostasis of the glutamatergic system [3]. During withdrawal these adaptive responses become visible. Indeed, microdialysis studies within the nucleus accumbens and the hippocampus, which were performed in alcohol-dependent rats after withdrawal, show that glutamate levels increase 2–3 fold approximately 6 h after withdrawal, which is the time associated with the commencement of seizures and hyperexcitability, and reach a peak at 12 h then decline to baseline values at 24–36 h from the interruption of the chronic

alcohol treatment. It is important to note that these changes in glutamate are observed in occurrence with overt physical withdrawal signs.

Molecular Mechanisms of Addictive Behavior

Chronic drug use and abuse leads to ►sensitization processes within the mesolimbic system. In the case of psychostimulants and opioids, in vivo measurements of extracellular dopamine levels provide direct evidence that these drugs, when administered under an intermittent injection schedule, can lead to a more pronounced increase in dopamine levels as compared to acute administration of these drugs. A major hypothesis postulates that the activation of a sensitized dopaminergic system (►Dopamine System) by conditioned stimuli is directly involved in drug craving [4]. Changes within the mesolimbic dopaminergic system and behavioral abnormalities that characterize drug addiction are long-lasting, and it is thought that regulation of neural gene expression is involved in the process by which drugs of abuse cause a state of addiction. The transcription factor Δ FosB represents one mechanism by which drugs of abuse can produce relatively stable changes in the brain that contribute to drug-induced sensitization processes and ►reinstatement of drug-seeking behavior in mice and rats. Δ FosB, a member of the Fos family of transcription factors, is induced in brain, in a region- and cell-type-specific manner by drugs of abuse. Thus, Δ FosB can be seen as a “molecular switch” that gradually converts acute drug responses into relatively stable adaptations that may contribute to the long-term neural and behavioral changes underlying addiction. However, Δ FosB plays also a role in long-term adaptive changes in the brain associated with other conditions, such as Parkinson’s disease, depression, and antidepressant treatment. Other transcription factors such as Period2 (Per2), are also induced by drugs of abuse but again various stressors and antidepressants can also induce a long-lasting up-regulation of Per2.

Following chronic drug intake long-term neuroplastic changes do not only occur in the brain reward system but also in brain stress systems that provide a negative motivational state that drives addictive behavior. This leads to further drug intake motivated by relief of negative emotionality. The changes in the reward and stress systems maintain hedonic stability in an ►allostatic state and one key mechanism in the transition to negative reinforcement is a recruitment of corticotropin-releasing factor (CRF) signaling within the ►extended amygdala. Thus long-term up-regulation of CRF1 receptors is observed in the amygdala following excessive chronic drug intake, and CRF1 receptor antagonists selectively block emotionality, excessive drug intake, and stress-induced reinstatement of drug-seeking behavior [5].

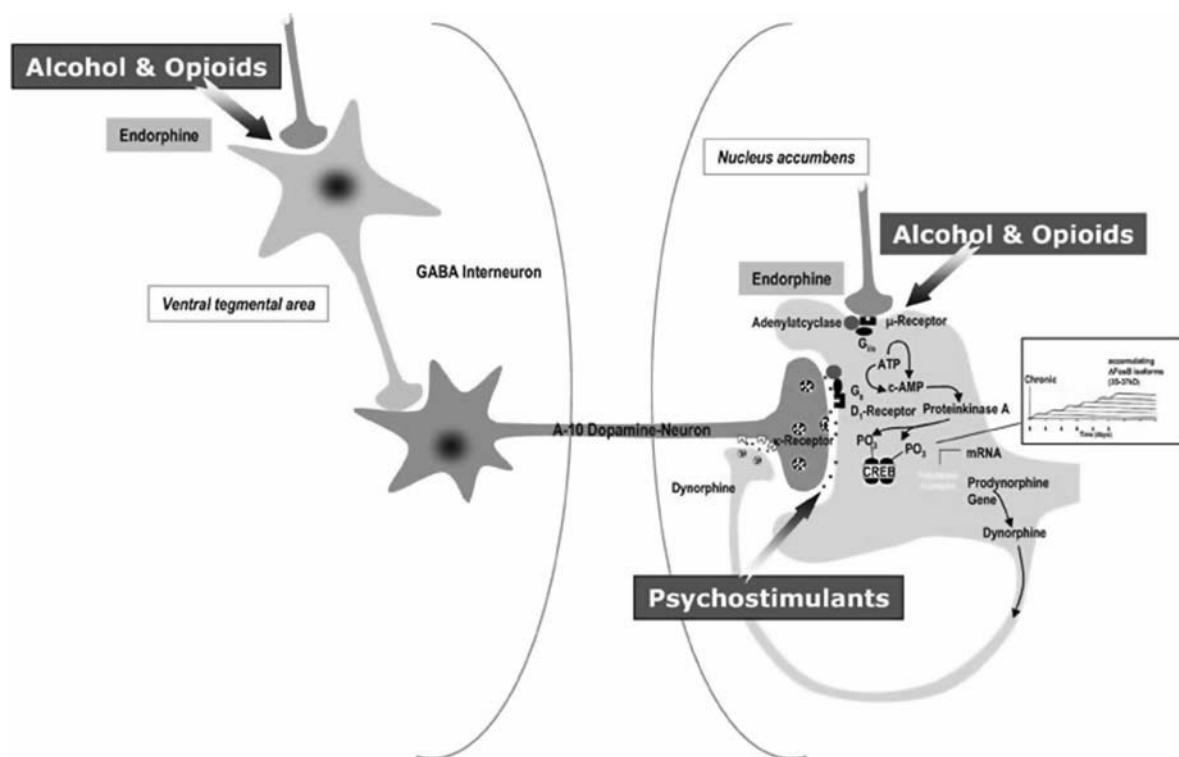
Psychostimulants and Opioids

Long-lasting alterations within the glutamatergic system seem to be critically involved in cocaine addiction. In particular, glutamatergic projections onto dopaminergic neurons (Fig. 1) within the ventral tegmental area undergo synaptic changes following cocaine administration. In the dopaminergic cells, cocaine and other psychostimulants influence at least two forms of synaptic plasticity linked to glutamatergic transmission, namely LTP and LTD. Thus, by increasing synaptic strength, facilitating LTP and/or reverting LTD processes these drugs augment glutamatergic input onto dopaminergic neurons and finally promote enhanced dopamine release in brain areas such as the nucleus accumbens and the prefrontal cortex. Along with those synaptic changes locomotor sensitization, reinforcement, and cocaine-seeking behavior occur— all phenomena that are considered as landmarks of addictive behavior in rodents. These behavioral phenomena may indeed be linked to the glutamatergic synapses. Thus, intra-VTA administered glutamate antagonists reduce locomotor sensitization, reduce cocaine, and opioid reinforcement

measured by conditioned place preference, and suppress drug-seeking behavior. The fact that in vivo cocaine administration enhances AMPA-synaptic transmission and increases the AMPAR:NMDAR ratio in dopaminergic cells led further to the conclusion that AMPA receptors located on these neurons could play a role in these and other manifestations of cocaine addiction.

Alcohol

Preclinical as well as clinical data strongly imply that craving and relapse for alcohol can be induced through different mechanisms. A first pathway may induce alcohol craving and relapse due to the mood enhancing, positive reinforcing effects of alcohol consumption. This pathway seems to involve opioidergic and dopaminergic systems in the ventral striatum. The role of the dopaminergic system may lie in the direction of attention toward reward-indicating stimuli, while the induction of euphoria and positive mood states may be mediated by opioidergic systems. Associative learning may, in turn, transform positive mood states and previously neutral environmental stimuli into



Drug Addiction/Dependence. Figure 1 Actions of drugs of abuse on dopaminergic neurons. Acute and long-lasting effects of alcohol, opioids, and psychostimulants on mesolimbic dopaminergic neurons. Nicotine, cannabinoids, and other drugs of abuse also affect these neurons in order to produce their reinforcing and long-lasting effects. Note that all drugs of abuse enhance either directly or indirectly dopamine release in the nucleus accumbens. Activation of neurons within the nucleus accumbens by chronic drug use leads to long-lasting changes in gene expression (e.g., prodynorphine gene) as indicated by accumulated Δ FosB isoforms (small inset).

alcohol-associated cues that acquire positive motivational salience and induce reward craving. A second and potentially independent pathway may induce alcohol craving and relapse by negative motivational states, including conditioned withdrawal and stress. This pathway seems to involve the glutamatergic system and the CRF-system. Chronic alcohol intake leads to compensatory changes within these systems. During withdrawal and abstinence increased glutamatergic excitatory neurotransmission as well as increased CRF release leads to a state of hyperexcitability that becomes manifest as craving, anxiety, and autonomic dysregulation [5]. Moreover, cues associated with prior alcohol intake that are not followed by actual drug consumption may induce ► **conditioned withdrawal**.

Pharmacological Relevance

Treatment of drug addicts can be separated into two phases: detoxification and relapse prevention. Detoxification programs and treatment of physical withdrawal symptoms, respectively, is clinically routine for most drugs of abuse. However, pharmacological intervention programs for relapse prevention are still not very efficient.

Detoxification

Alcohol

Cessation of prolonged heavy alcohol abuse may be followed by alcohol withdrawal or life-threatening alcohol withdrawal delirium. Typical withdrawal symptoms are autonomic hyperactivity, increased hand tremor, insomnia and anxiety, and are treated with benzodizepines and thiamine. Alcoholism is the most common cause of thiamine deficiency and can lead in its extreme form to the ► **Wernicke's syndrome** that can be effectively treated by high doses of thiamine.

Opioids

Opiate overdose is a medical emergency that can result in respiratory and CNS depression. The opioid receptor antagonist naloxone immediately reverses cardiorespiratory depression. However, repeated naloxone administration is required, since the effects of naloxone last for 30 min, while opioid agonists can remain at potentially lethal blood levels for several hours.

Relapse Prevention

Alcohol

Anti-relapse compounds have recently been registered for relapse prophylaxis in weaned alcoholics in various European countries and in the United States. Acamprosate, the Ca^{2+} salt of *N*-acetyl-homotaurinate, interacts with the glutamatergic system in various brain regions and reduces Ca^{2+} fluxes through voltage-dependent ion channels. The opioid receptor antagonist naltrexone interferes with alcohol-induced reinforcement via blockade of opioid receptors. Although the dopaminergic

system has been in the focus of alcohol research for many years, clinical trials interfering with several components of this system displayed rather disappointing results. This situation, however, could change in light of the discovery that dopamine D3 receptor antagonism produces very consistent and robust results in preclinical studies. CRF1 receptor signaling and the endocannabinoid system integrate stress-related events and thereby mediate relapse behavior. Beside CRF1 receptor and CB1 receptors many new targets have been identified and several new compounds are currently undergoing clinical testing. However, given the heterogeneity in treatment response, genetic and protein markers as well as endophenotypes are currently characterized for individualized pharmacotherapy.

Opioids

Substitution therapy with methadone or buprenorphine has been very successful in terms of harm reduction. Some opiate addicts might also benefit from naltrexone treatment. One idea is that patients should undergo rapid opiate detoxification with naltrexone under anaesthesia, which then allows further naltrexone treatment to reduce the likelihood of relapse. However, the mode of action of rapid opiate detoxification is obscure. Moreover, it can be a dangerous procedure and some studies now indicate that this procedure can induce even more severe and long-lasting withdrawal symptoms as well as no improvement in relapse rates than a regular detoxification and psychosocial relapse prevention program.

- Opioid System
- GABAergic System
- Dopamine System
- Tolerance and Desensitization
- Ethanol
- Psychostimulants
- Psychotomimetic Drugs

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Drug Discovery

Drug discovery is the identification and optimization of compounds for further development of drugs.

- High-throughput Screening
- Combinatorial Chemistry
- Orphan Receptors

Drug–Drug Interaction

- Drug Interaction

Drug Efficacy

Drug efficacy refers to the capacity of a drug to produce an alteration in a target cell/organ after binding to its receptor. A competitive antagonist, that occupies a binding site without producing any alteration in the receptor, is considered to have an efficacy of zero. The efficacy is generally independent of potency/affinity, and is related to the maximum effect that a particular drug is capable of producing.

- Drug–Receptor Interaction

Drug Interactions

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Synonyms

Drug–drug interactions; Pharmacokinetic and/or pharmacodynamic interactions; Pharmacokinetic and/or pharmacodynamic consequence of multiple drug therapy

Definition

Drug interactions can occur when two drugs are administered together, one affecting the pharmacological and/or adverse effects of the other. One or both of the drugs could be affected leading to either clinically beneficial interactions (e.g. increased pharmacological effects or reduced adverse effects) or harmful interactions (e.g. reduced pharmacological effects or increased adverse effects).

Basic Mechanisms

Pharmacokinetic Interactions

Interactions resulting from a change in the amount of drug reaching the site of action are called pharmacokinetic interactions (Fig. 1). A co-administered drug can affect any of the processes of absorption, distribution, metabolism, and excretion of the original drug, which are determinants of its pharmacokinetic profile [1–3].

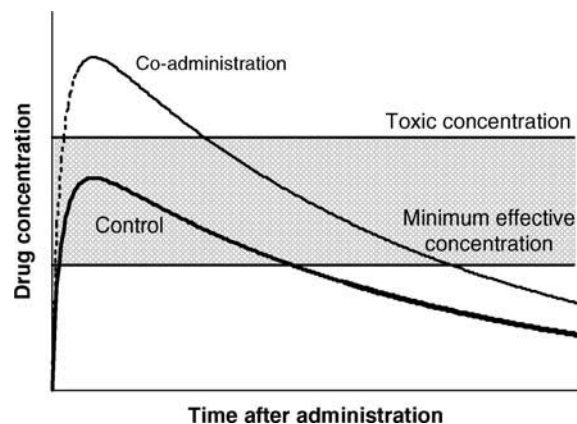
Drug Interactions During Absorption

Changes in Gastrointestinal pH

Following oral administration, drug molecules in their lipophilic non-ionic form are more easily absorbed by ► [simple diffusion](#) through the gastrointestinal mucosa. Therefore, the alteration in the fraction of non-ionic form caused by gastrointestinal pH change due to co-administered drugs, such as antacids, can lead to a change in drug absorption.

Absorption, Chelation, and Complex Formation

Charcoal, which is used as a detoxicant in overdose, etc., is able to adsorb drugs, thereby causing a reduction in drug absorption. Absorption of new quinolone antibiotics is reduced by forming an insoluble chelate with secondary or tertiary cations, such as Mg^{2+} or Al^{3+} ,



Drug Interactions. Figure 1 Increase in drug concentration caused by pharmacokinetic interactions. Shadow represents the therapeutic range.

found in many antacids. The cholesterol-lowering agent, cholestyramine, which is an anion-exchange resin, reduces the absorption of drugs like warfarin by binding to them.

Changes in Gastrointestinal Motility

Because the majority of orally administered drugs are absorbed mainly from the upper part of the small intestine, an alteration in gastric emptying rate (The rate of orally administered drug reaching the small intestine from the stomach. This can be affected by various factors such as food, body position, and certain drugs.) caused by co-administered drugs can lead to alterations in drug absorption. For example, anticholinergic drugs, such as propantheline, delay gastric emptying by reducing gastrointestinal motility, causing the reduced absorption rate of acetaminophen, etc., while metoclopramide has the opposite effect.

Effects on Transporters such as P-Glycoprotein

P-glycoprotein (P-gp) works as a transporter at the intestinal mucosa pumping drugs out into the lumen. Absorption of P-gp substrates, such as digoxin, cyclosporine, etc., can be increased by inhibitors of P-gp and reduced by inducers.

Drug Interactions During Distribution

Displacement from Plasma Protein Binding Sites

In general, only the free molecules which are not bound to plasma proteins, such as albumin and α_1 -acid glycoprotein, cross biological membranes and exhibit pharmacological effects. Therefore, displacement from plasma protein binding sites by co-administered drugs can cause an increase in the unbound fraction of the original drug in plasma, possibly leading to increased pharmacological effects.

Effects on Transporters

The tissue distribution of substrates of transporters involved in the **active transport** into or out of tissues

such as liver, kidney, brain, tumour cells, etc., can be affected by the co-administration of inhibitors and/or inducers of such transporters. For example, cyclosporin A inhibits the hepatic uptake of cerivastatin via OATP-C/OATP2, a transporter localized on the sinusoidal membrane of the liver. Quinidine inhibits the brain efflux of loperamide via P-gp, thereby enhancing its distribution into the brain.

Drug Interactions During Metabolism

Enzyme Inhibition

Following concurrent administration of two drugs, especially when they are metabolized by the same enzyme in the liver or small intestine, the metabolism of one or both drugs can be inhibited, which may lead to elevated plasma concentrations of the drug(s), and increased pharmacological effects. The types of enzyme inhibition include reversible inhibition, such as **competitive** or **non-competitive inhibition**, and irreversible inhibition, such as **mechanism-based inhibition**. The clinically important examples of drug interactions involving the inhibition of metabolic enzymes are listed in Table 1 [1, 4].

Enzyme Induction

The co-administration of drugs which induce the metabolic enzymes in the liver or small intestine can reduce the plasma concentrations of drugs which are substrates of the enzyme, leading to reduced drug effects. For example, the plasma concentrations of many drugs which are substrates of the enzyme CYP3A4, such as cyclosporine, are decreased by co-administration of rifampicin, which is an inducer of CYP3A4.

Drug Interactions During Excretion

Changes in Urinary pH

As in gastrointestinal absorption, the lipophilic non-ionic form of a drug is more susceptible to reabsorption from the renal tubules by simple diffusion. Therefore,

Drug Interactions. Table 1 Examples of clinically important drug interactions due to enzyme inhibition

Substrate	Inhibitor	Inhibited enzyme	Possible clinical outcome
Theophylline	Ciprofloxacin, fluvoxamine, etc.	CYP1A2	Theophylline toxicity
Phenytoin	Chloramphenicol, isoniazid, etc.	CYP2C9, CYP2C19	Phenytoin intoxication
Tolbutamide	Sulfaphenazole	CYP2C9	Hypoglycaemia
Warfarin	Fluconazole, metronidazole, etc.	CYP2C9	Haemorrhage
Astemizole, terfenadine	Ketoconazole, erythromycin, etc.	CYP3A4	Ventricular, arrhythmia
Cyclosporine, tacrolimus	Ketoconazole, erythromycin, etc.	CYP3A4	Cyclosporine/tacrolimus toxicity
Lovastatin, simvastatin	Ketoconazole, erythromycin, etc.	CYP3A4	Rhabdomyolysis
Azathiopurine, mercaptopurine	Allopurinol	Xanthine oxidase	Azathiopurine/mercaptopurine toxicity

reabsorption of weakly acidic drugs can be enhanced (or inhibited) by co-administration of drugs which reduce (or elevate) the urinary pH, leading to elevated (or reduced) plasma concentrations. The opposite effects are observed for the weakly basic drugs.

Effects on Renal Tubular Secretion

The co-administration of drugs which inhibit the transporters involved in renal tubular secretion can reduce the urinary excretion of drugs which are substrates of the transporter, leading to elevated plasma concentrations of the drugs. For example, probenecid increases the plasma concentration and the duration of effect of penicillin by inhibiting its renal tubular secretion. It also elevates the plasma concentration of methotrexate by the same mechanism, provoking its toxic effects.

Effects on Biliary Excretion

The co-administration of drugs which inhibit the transporters involved in biliary excretion can reduce the biliary excretion of drugs which are substrates of the transporter, leading to elevated plasma concentrations of the drugs. For example, biliary and urinary excretion of digoxin, both mediated by P-gp, are inhibited by quinidine which is an inhibitor of P-gp.

Pharmacodynamic Interactions

Pharmacodynamic interactions are drug interactions involving alterations in drug effects following co-administration of drugs, without alterations in the drug concentrations at the site of action. They include direct interactions, such as two drugs exhibiting their pharmacological effects via binding to the same receptor, and indirect interactions, in which the drug effects are affected by biochemical or physiological changes due to the co-administered drug.

Additive or Synergistic Interactions

When two drugs with similar pharmacological and/or adverse effects are administered simultaneously, an additive or synergistic increase in their effects can be observed. These include the increased sedative effects seen in the central nervous system following co-administration of alcohol and benzodiazepine hypnotics/anxiolytics and the increased risk of bleeding following co-administration of the anticoagulant, warfarin, and the non-steroidal anti-inflammatory drug, aspirin.

Antagonistic Interactions

The pharmacological and/or adverse effects of a drug can be reversed by co-administration of drugs which compete for the same receptor. For example, an opioid receptor antagonist naloxone is used to reverse the effects of opiates. Drugs acting at the same site with opposite effects also can affect each other, e.g. the reduction in the anticoagulant effect of warfarin by vitamin K.

Pharmacological Relevance

Drug interactions can cause serious problems in clinical practice especially when the affected drug has the potential to be highly toxic. Furthermore, pharmacokinetic interactions are clinically important if the affected drug has a narrow therapeutic range (i.e. small difference between the minimum effective concentration and the toxic concentration; Fig. 1) and a steep concentration–response curve (i.e. significant alterations in pharmacological and/or adverse effects caused by small changes in blood concentration).

Although drug interactions involving plasma protein binding and drug metabolism are often evaluated in *in vitro* studies, the interactions observed *in vitro* are not necessarily observed *in vivo* or clinically relevant. For example, even when the plasma unbound fraction of a drug is increased by protein binding displacement, kinetic theory (clearance concept) indicates that the steady-state plasma unbound concentration may not change because the unbound drug is subject to metabolism and excretion. Therefore, this type of drug interaction is unlikely to be clinically significant unless a high clearance drug is administered intravenously. However, a transient increase in the plasma unbound concentration can be observed under non-steady-state conditions, especially for drugs with small volumes of distribution. It should be taken into account that such a transient increase may cause some side effect of drugs.

In the case of drug interactions involving metabolic inhibition, little increase in the substrate concentration is expected when the inhibition constant (K_i) determined in *in vitro* studies using human liver samples is larger than the inhibitor concentration *in vivo*. Various approaches have been adopted using mathematical models in attempts to quantitatively predict *in vivo* drug interactions from *in vitro* data [5].

► Drug–Receptor Interaction

► Pharmacokinetics

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Drug Metabolism

The metabolism of foreign compounds (xenobiotics) often takes place in two consecutive reactions, classically referred to as phases one and two. Phase I is a functionalization of the lipophilic compound that can be used to attach a conjugate in Phase II. The conjugated product is usually sufficiently water-soluble to be excretable into the urine. The most important biotransformations of Phase I are aromatic and aliphatic hydroxylations catalyzed by cytochromes P450. Other Phase I enzymes are for example epoxide hydrolases or carboxylesterases. Typical Phase II enzymes are UDP-glucuronosyltransferases, sulfotransferases, N-acetyltransferases and methyltransferases e.g. thiopurin S-methyltransferase.

►p450 Mono-Oxygenase System

Drug Receptor Theory

►Drug–Receptor Interaction

Drug Reinforcement

In its reinforcing capacity, a drug of abuse increases the frequency of preceding responses, and accordingly is called a reinforcer. All drugs of abuse are primary reinforcers and lead to self-administration.

►Drug Addiction/Dependence

Drug–Receptor Interaction

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Synonyms

Drug receptor theory; Quantification of drug effect

Definition

Mathematical models of the interaction between drugs and ►receptors, based on ►Michaelis–Menten kinetics, are utilized to create the quantitative tools currently used in receptor pharmacology to quantify drug effect in biological systems. Such tools are necessary since drugs almost always are tested in surrogate systems until they are known to be sufficiently active and safe for therapeutic use. The aim of receptor pharmacology is to define the molecular properties of ►affinity and ►intrinsic efficacy of drugs; these can be used to predict drug effect across different biological systems. It will be seen that the properties of affinity and efficacy are sufficient to describe the case of the interaction of a single molecule, target receptor, and cell. For an extended system of two interacting molecules (e.g., agonist and antagonist), target receptor and cell, it is useful to know additional information, namely knowledge as to whether the interaction is orthosteric or allosteric, and (optionally) the kinetics of interaction of the molecules with the target (target residence time) in order to fully characterize drug activity.

Basic Mechanisms

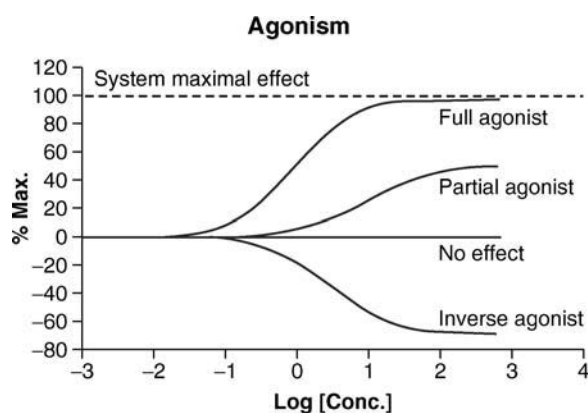
A basic premise in receptor pharmacology is that all drugs have affinity for receptors (the chemical property that unites the drug with the receptor), and some drugs have efficacy, the chemical property that causes the receptor to change its behavior toward its host cell. Drugs that have efficacy can produce concentration-dependent responses in physiological systems, characterized by a concentration–response curve (also often referred to as a dose–response curve).

Drugs that produce pharmacological activation of a system are called *agonists*, those that inhibit activation of a receptor system are called *antagonists*, and those that reverse spontaneously active receptor systems (►constitutive receptor activity) are called *inverse agonists* (Fig. 1). This latter class of drugs reduce elevated basal responses. Agonism is the observed effect of a ligand producing stimulus to a receptor. The host cellular system processes that stimulus and yields an observable response.

The common currency of drug receptor pharmacology is the dose–response curve, as it defines the relationship between concentrations of drug and the resulting effect. Dose–response curves have three basic properties with which they can be described: threshold abscissal value, slope, and maximum asymptote. The location parameter of the dose–response characterizes drug potency; most often this is quantified as the EC₅₀ or the molar concentration of drug producing half the maximal effect. Given tissue systems have maximal capabilities to return drug response. When an agonist produces a maximal response that is equal to the system maximal response it is referred to as a *full agonist*.

(Fig. 1). If an agonist produces a submaximal system response it is called a *partial agonist* (Fig. 1). While the potency of an agonist is quantified by the location parameter of the dose-response curve (EC_{50}), a reflection of (but not a direct measure of) the intrinsic efficacy of an agonist is given by its maximal response.

It is important to note that both the quality and quantity of biological response obtained with a given drug depends very much on the assay system used to make the measurement. If the assay does not have the means to detect a given efficacy, then none will be observed; this should not be taken to mean that the drug does not have that particular efficacy. A common case in point

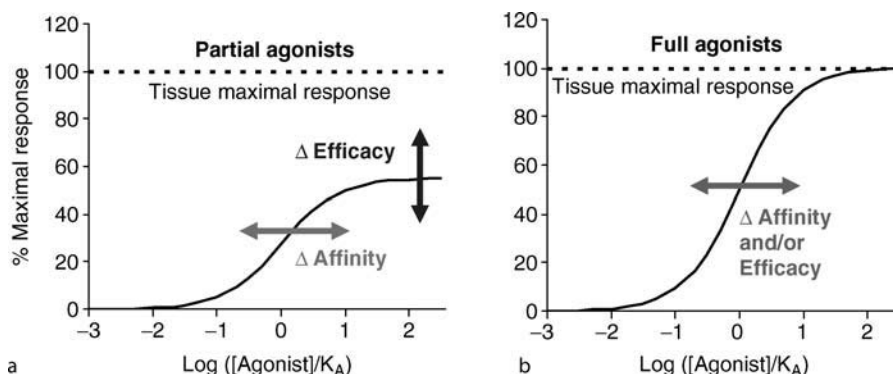


Drug-Receptor Interaction. Figure 1 Dose-response curves to drugs that have direct effects on physiological systems. A drug that produces the maximal effect capable from the system is a *full agonist*. A submaximal effect is produced by a *partial agonist*. Drugs may not produce directly observable effects but may be present on the receptor to produce antagonism (see Fig. 3). If a system is constitutively active and shows an actively elevated basal response, then a drug that reduces this is termed an *inverse agonist*.

is the failure of nonconstitutive receptor systems to detect negative efficacy (inverse agonism). For this particular efficacy to be detected, an especially engineered assay must be used. Similar effects are observed in the receptor-mediated signaling effects. While most agonist-bound receptor activates G-proteins, some initiate the binding of intracellular proteins such as beta-arrestin to induce kinase signaling. There are cases, such as for the β -adrenoceptor antagonist ICI 118,551, where the same drug will have a negative efficacy for one response system (i.e., G-proteins) and positive efficacy for another (beta-arrestin kinase stimulation).

In general, if a drug generates a direct response in a system, and if that response is lower in magnitude than the system maximal response (i.e., it is a partial agonist, see Fig. 1), then the location of the concentration-response curve along the concentration axis is determined by the affinity of the agonist and the maximal response is determined by the magnitude of the efficacy (Fig. 2a). If the agonist produces the full system maximal response (it is a full agonist), then it is not possible to assess efficacy (since the maximal response is constant) and the location of the concentration-response curve is determined by a complex function of both affinity and efficacy (Fig. 2b).

The inhibition of agonist response is termed antagonism. The effect that a given antagonist has on the dose-response curve to an agonist can be a clue to the mechanism of action of that antagonists' interaction with the receptor. In the presence of an antagonist, more agonist must be present in the receptor compartment to produce a response than would be necessary in the absence of the antagonist. A singular characteristic of an antagonist is its effect on the maximal capability of an agonist to overcome the presence of the antagonist and produce the system maximal response. If enough agonist can be added to produce the agonist maximal response in the presence of the antagonist, the



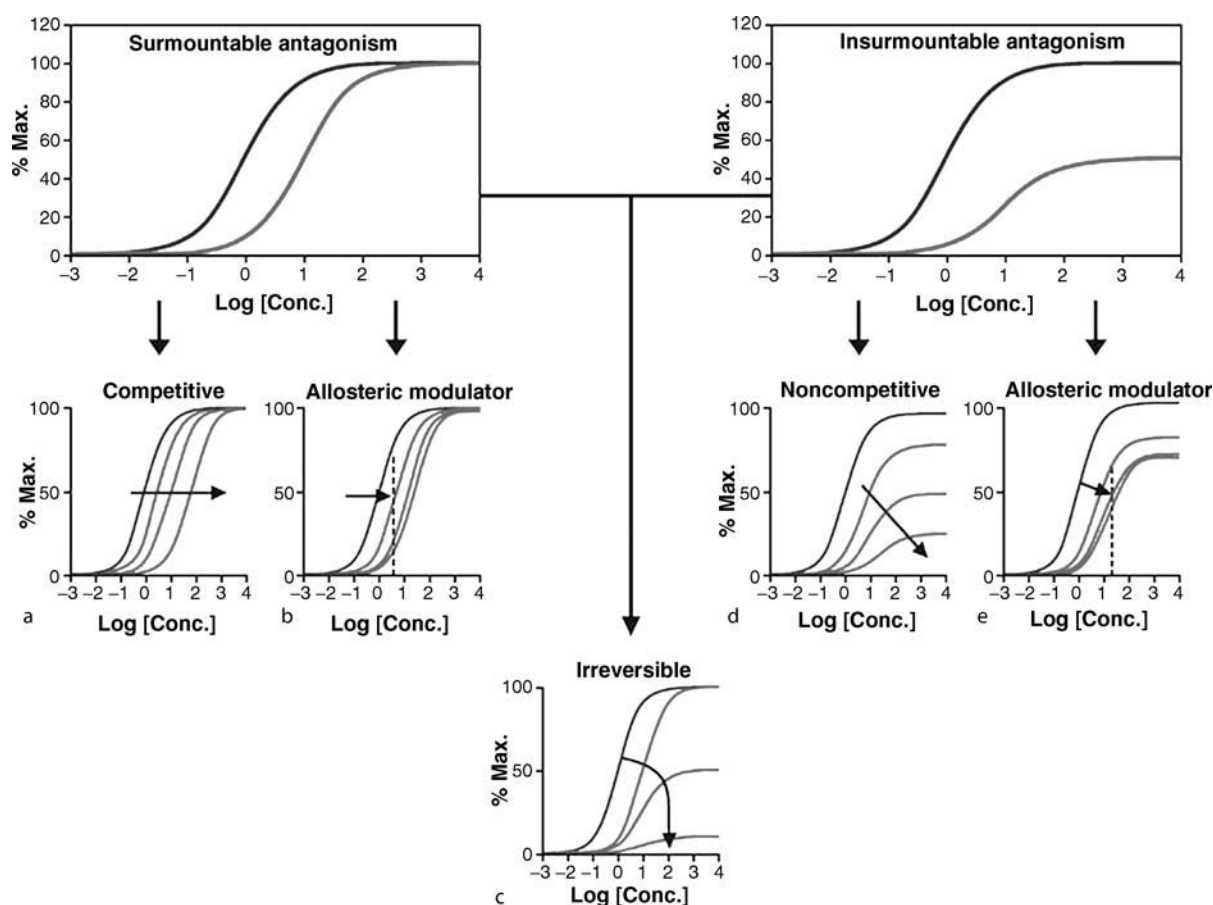
Drug-Receptor Interaction. Figure 2 Relationships between affinity and efficacy with different agonist response patterns. (a) For partial agonists, differences in maximal responses between agonists relate to differences in efficacy. Differences in the location parameter of the concentration-response curve (potency) indicate differences in affinity. (b) For full agonists, differences in potency indicate differences in either affinity, efficacy or both.

antagonism is referred to as surmountable antagonism (see Fig. 3). If no amount of agonist will produce the maximal response, the antagonism is referred to as insurmountable (see Fig. 3).

A basic concept in receptor pharmacology is the idea of orthosteric and allosteric interaction. Orthosteric interaction occurs when two molecules compete for a single binding domain on the receptor. With allosteric interactions two molecules each have their own binding domain on the receptor and the two interact through effects on the protein (conformational change). Thus, with orthosteric interactions only one molecule may occupy the receptor at any one instant whereas with allosteric interactions both molecules can bind to the receptor at the same time. There are implications for

pharmacological activity, especially for antagonists, that arise from these two molecular mechanisms (*vide infra*).

There are certain molecular mechanisms of antagonism associated with these observed patterns on dose-response curves. Thus, ►**competitive antagonists** produce parallel shifts to the right of agonist dose-response curves with no diminution of maximal response through an orthosteric interaction of antagonist and agonist (Fig. 3a). Theoretically, there is no limit to the degree of dextral displacement a given competitive antagonist can produce on a dose-response curve. ►**Schild analysis** is used to measure the affinity of competitive antagonists. This same pattern of response also can occur with ►**allosteric modulators** (Fig. 3b) but in this case the shift of the agonist dose-response curve



Drug-Receptor Interaction. Figure 3 Various patterns of antagonism of drug effect. Antagonism is classified according to effects on the dose-response curve to the agonist (blue lines). Two general classifications are surmountable antagonism (maximal response to the agonist retained – top left panel) or nonsurmountable antagonism (depressed maximal response to the agonist – top right panel). These general patterns can be the result of different molecular mechanisms. (a) Orthosteric competition between the agonist and antagonist for the same binding site on the receptor. (b) Binding of an allosteric modulator to its own binding site to modify the affinity or efficacy of the agonist in a saturable manner. (c) Irreversible blockade of receptors in a system with *receptor reserve* (parallel shift to the right followed by depression of the maximal response at higher concentrations of antagonist). (d) Blockade of receptor function or access of agonist to the receptor either orthostERICALLY or allosterically in a system with little receptor reserve for the agonist. (e) Saturable allosteric modulation of receptor function by an antagonist in a system with little receptor reserve for the agonist.

is limited to a maximal value defined by the molecular ►co-operativity factor of the antagonist. Thus, a hallmark of allosteric inhibition is that it is saturable and reaches a maximal asymptotic value. In some cases, ►irreversible antagonists can produce parallel shifts to the right of dose-response curves if there is a ►receptor reserve for the agonist (Fig. 3c). This latter mechanism can be detected with increasing concentrations of irreversible antagonist since these eventually cause depression of the maximal response.

In cases where insurmountable antagonism is observed, this can be evidence of ►non-competitive antagonism. This can result from an inhibition of receptor function (either orthosteric or allosteric) or be due to antagonist-mediated modification of receptor reactivity to the agonist (Fig. 3e). The *method of ►Gaddum* is used to measure the affinity of noncompetitive antagonists for receptors. Parenthetically, similar effects on dose-response curves are produced by irreversible antagonists when there is no receptor reserve for the agonist.

While antagonist potency (quantified as the equilibrium dissociation constant of the antagonist-receptor complex, specifically the rate of offset of the antagonist away from the receptor divided by the rate of onset toward the receptor) usually is used as a measure of the therapeutic utility of an antagonist (i.e., high potency leads to the use of low dose with the concomitant avoidance of high concentrations that may produce side effects), the kinetics of receptor antagonism also are important. Thus, if antagonist negation of receptor function is the desired effect, then for two antagonists of equal potency, the one with the most persistent binding (slowest rate of offset from the receptor) is preferred. This is referred to as target residence time.

Pharmacological Relevance

An important tenet of receptor pharmacology states that the molecular properties of a drug, namely affinity and intrinsic efficacy, are interpreted and reflected by physiological systems and that this process *controls* what is observed as drug effect. For example, a physiological system requires a certain sensitivity to return response from stimulation by a weak agonist, i.e., the receptor coupling of that system must be of sufficient efficiency to amplify the stimulus into an observable response. When the same weak agonist is tested in a physiological system of lower sensitivity, it might be an antagonist. Thus, it can be seen that the monikers of agonist and antagonist can be system dependent and unreliable as molecular labels for drugs. In the same manner that a system must be of sufficient sensitivity to detect efficacy and return response, it also can be overloaded by a strong stimulus. When this occurs, the system returns the system maximal response and the agonist demonstrates full agonism. A series of agonists of differing intrinsic efficacy may all overload

a given system and all return the same (system) maximal response (all be full agonists); this does not imply that these agonists are of equal efficacy but only that the system was unable to discern different efficacies beyond a certain level. For this reason, the labels of full and partial agonist are also system dependent and not useful for molecular characterization of drugs.

Since drugs are tested in many surrogate systems, it is necessary to develop methods to quantify drug effect in a system-independent manner. Absolute scales are not practical, or in some cases, even possible, in this process. As discussed previously, while an absolute potency for an agonist can be determined by the EC_{50} , the magnitude of this value depends on the sensitivity of the particular measuring system and thus it cannot be extrapolated to other systems. Rather, the relative potency of agonists (ratios of EC_{50} values) is used to quantify agonist power to induce response. This process utilizes the null method and isolates only the intrinsic ability of the agonists to produce response at the receptor level. This allows for the resulting potency ratios to be a measure of relative agonist activity that is comparatively independent of the system in which the measurement is made. This ratio transcends the particular system in which it is measured and is applicable to all systems in which the agonists produce maximal response. Under optimal conditions, the therapeutic profile of the standard agonist will be known in humans, therefore the agonist potency ratio can be used to gauge the expected activity of the experimental agonist in the therapeutic arena.

In the case of antagonists, absolute measures of potency are theoretically possible since these are chemical terms describing the affinity of the drugs for receptor protein. However, physiological systems can also control the observed antagonism. For example, a noncompetitive antagonist will produce a diminution of the maximal response to an agonist in a system where the response is linearly related to the receptor occupancy (Fig. 3d). However, if the receptors in a system are coupled with high efficiency and the agonist has high efficacy, then maximal responses may be achieved with less than maximal agonist receptor occupancy, i.e., there may be a ►receptor reserve for the agonist. Under these circumstances the agonist may still produce the maximal response even when the antagonist completely inactivates a portion of the receptors. When this occurs the antagonism resembles competitive antagonism at low concentrations and noncompetitive antagonism at higher concentrations (resembling the profile in Fig. 3c). This would be system dependent and not necessarily indicative of the molecular mechanism of the antagonist.

Another example of where the setpoint of the physiological system can change the observed behavior of drugs is the absence of direct effects of inverse agonists in nonconstitutively active receptor systems.

A receptor system must be constitutively active (elevated basal response) to detect inverse agonism (Fig. 1). In nonconstitutively active receptor systems, inverse agonists behave as simple competitive antagonists.

In general, receptor theory uses indirect mathematical models to estimate descriptors of drug effect. These descriptors still must be used with the proviso that biological systems may still modify drug effect in a system-dependent manner and thus predictions of therapeutic effect must be made with caution across different systems.

- Drug Interactions
- G-protein-coupled Receptors
- Tolerance and Desensitization

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Dubin–Johnson Syndrome

The Dubin–Johnson Syndrome is a rare hereditary disease, which is associated with hyperbilirubinemia (high bilirubin and bilirubin-glucuronide plasma concentrations). The disease is caused by *loss of function* mutations in the *ABCC2* gene, which is coding for the ABC-transporter MRP2 (also termed as *multispecific organic anion transporter* (MOAT)). Normally, this transporter is localized in the canalicular membrane of hepatocytes, where it eliminates bilirubin-glucuronide and other organic anions into the bile.

- ABC-Transporter
- MDR-ABC-Transporters

Dynamic Programming

In general, dynamic programming is an algorithmic scheme for solving discrete optimisation problems that have overlapping subproblems. In a dynamic

programming algorithm, the definition of the function that is optimised is extended as the computation proceeds. The solution is constructed by progressing from simpler to more complex cases, thereby solving each subproblem before it is needed by any other subproblem. In particular, the algorithm for finding optimal alignments is an example of dynamic programming.

- Bioinformatics

Dyskinesias

Dyskinesias are abnormal movements, usually caused by neurological diseases or by drugs used to treat neurological (e.g., levodopa) or psychiatric diseases (e.g., neuroleptics).

- Anti-Parkinson Drugs
- Antipsychotic Drugs
- Tardive Dyskinesia
- Catechol-O-Methyltransferase and its Inhibitors

Dyslipidemia

Dyslipidemia is change in the normal lipid concentrations in the blood. In particular, hypercholesterolemia is a major cause of increased atherogenic risk, leading to atherosclerosis and atherosclerosis-associated conditions, such as coronary heart disease, ischemic cerebrovascular disease and peripheral vascular disease. Both genetic disorders and diets enriched in saturated fat and cholesterol contribute to the elevated lipid levels in a considerable part of the population of developed countries. Hypertriglyceridemia, when severe, may cause pancreatitis. Moderately elevated levels of triglycerides are often associated with a syndrome distinguished by insulin resistance, obesity, hypertension and substantially increased risk of coronary heart disease. Hypercholesterolemia, especially, requires treatment either by diet and/or with lipid-lowering drugs (e.g. statins, anion exchange resins).

- Statins
- Anion Exchange Resins
- Fibrates

Dysrhythmias

- Antiarrhythmic Drugs

E3 Ligase

E3 ligases are enzymes that together with E1 and E2 ligases catalyse the transfer of the ubiquitin polypeptide onto proteins destined for degradation. E1 ligase activates ubiquitin, E2 ligase performs the actual transfer of ubiquitin to the target protein, and E3 ligases are involved in substrate recognition and determining the specificity. Poly-ubiquitinated proteins are degraded in proteasomes.

- Ubiquitin/Proteasome
- SUMOylation
- Cadherins/Catenins

EC₅₀

The concentration of the drug at which it is half maximally effective in a certain preparation.

- Drug Receptor Interaction

ECE

Endothelin Converting Enzyme.

- Endothelins

Ecogenetics

Ecogenetics is the study of genetically determined inter-individual variation within one species with respect to the response to environmental chemicals or physical environmental factors.

- Pharmacogenetics

Ecstasy

Methylenedioxymethamphetamine.

- Psychostimulants

Ectodermin

A Ring-type ubiquitin ligase for Smad4, serving as a vital regulator of the transforming growth factor-beta (TGF-beta)/BMP signaling pathway in early embryonic development and cancer. As Smad4 is the Comediator Smad and facilitates the translocation of the R-Smads into the nucleus, ectodermin forces Smad4 out of the nucleus and promotes its degradation. Through this regulation of the TGF-beta signaling, ectodermin keeps ectoderm cells pluripotent until gastrulation and ensures that ectoderm cells do not undertake mesoderm fate. Ectodermin is also important in reducing TGF-beta/Smad4 induced cytostasis as it is expressed in the stem cells of colorectal cancer and intestinal crypts.

- Transforming Growth Factor-Beta
- Ubiquitin/Proteasome

Ectonucleotidase

An enzyme on the cell surface that metabolizes nucleotide derivatives by hydrolytically cleaving one or more phosphate groups.

- Adenosine Receptors

ED₅₀

The ED₅₀ is the dose of a drug required to achieve a half-maximal effect.

► Drug–Receptor Interaction

Edema

Edema refers to an accumulation of interstitial fluid to a point where it is palpable or visible. In general this point is reached with a fluid volume of 2–3 L. Edema formation is the result of a shift of fluid into the interstitial space due to primary disturbances in the hydraulic forces governing transcapillary fluid transport, and of subsequent excessive fluid reabsorption by the kidneys. Deranged capillary hydraulic pressures initiate edema formation in congestive heart failure and liver cirrhosis, whereas a deranged plasma oncotic pressure is leading to edema in nephrotic syndrome and malnutrition. Increased capillary permeability is responsible for edema in inflammation and burns.

► Diuretics

Edg Receptors

Edg receptors are a group of recently discovered ►G-protein coupled receptors, which mediate the action of lysophospholipids (sphingosine-1-phosphate, lysophosphatidic acid).

► Lysophospholipids

EEG

EEG is the abbreviation for ►Electroencephalogram.

EF-hand

A Ca²⁺-binding motif found in many Ca²⁺-binding proteins.

► Ca²⁺-Binding Proteins
► IP₃ Receptors
► S100 Proteins

Effector

Receptors induce a signal transduction process upon binding of an extracellular signal. The receptor-protein domain or receptor-linked protein whose activity is altered in order to generate the earliest signals in a signaling cascade are called effector. Enzymes or ion channels can be receptor-controlled effectors.

► Transmembrane Signaling

Efferent Function of Sensory Nerves

This indicates the unique property of capsaicin-sensitive primary afferent neurons to release mediators (neuropeptides and others) from both peripheral and central nervous system terminals upon adequate stimulation. Capsaicin and other chemical (protons) or physical (heat) stimuli release mediators from both peripheral and central nervous system terminals of these neurons. Capsaicin-induced release of mediators is fundamentally tetrodotoxin resistant despite being nerve mediated.

► Tachykinins and their Receptors

Efficacy

Efficacy is a parameter which describes the “strength” of a single drug-receptor complex in evoking a response from the cell or tissue. The ►intrinsic efficacy of a drug is a proportionality constant that defines the power of the drug to induce a response.

► Potency
► Drug–Receptor Interaction

EGF

Epidermal Growth Factor.

► Growth Factors

Eicosanoid

Any of the collection of oxygenated metabolites of arachidonic acid that are the product of cyclooxygenase, cytochrome P450, or lipoxygenase pathways.

► Prostanoids
 ► Leukotrienes
 ► Cyclooxygenases
 ► p450 Mono-oxygenase System

Elastase-like Proteinases

Elastase-like proteinases are serine proteinases that recognized peptide residues with linear aliphatic side chains (alanyl, valyl, leucyl or isoleucyl residues) and that effect hydrolysis of the polypeptide chain on the carboxy-terminal side of these residues. Examples of elastase-like proteinase are: pancreatic elastase, neutrophil elastase and proteinase-3.

► Non-viral Peptidases

Electrochemical Driving Force

The net electrochemical driving force is determined by two factors, the electrical potential difference across the cell membrane and the concentration gradient of the permeant ion across the membrane. Changing either one can change the net driving force. The membrane potential of a cell is defined as the inside potential minus the outside, i.e. the potential difference across the cell membrane. It results from the separation of charge across the cell membrane.

► Depolarisation

Electroencephalogram (EEG)

A measurement of the brain's electrical activity, usually measured by means of electrodes placed on the individual's scalp. The EEG is used clinically to differentiate arousal states and to diagnose epileptic phenomena. It can also be used for more advanced applications such as mapping areas of cognitive activity. By employing mathematical analysis (Fourier transform) of the EEG, a power spectrum can be generated. This yields a portrayal of the relative proportion of the various frequency components present in the signal, which are commonly divided into the following bands: DELTA less than 4 Hz; THETA 4–8 Hz; ALPHA 8–12 Hz; BETA 13–30 Hz; GAMMA greater than 30 Hz.

► Sleep
 ► Benzodiazepines
 ► Antiepileptic Drugs

Electromyogram (EMG)

Measurement of muscle activity, usually measured by electrodes placed on the skin. The EMG is used in sleep research to aid in the discrimination of sleep stages, and also as part of diagnosis of sleep disorders such as periodic limb movements and restless legs syndrome.

► Sleep

Electro-oculogram (EOG)

Measurement of movement of the eyeballs by means of electrodes placed adjacent to the eye sockets. The EOG parameter is important in defining ► REM sleep. Indeed, in animals sleep experiments where EOG data are not collected, the corresponding sleep state is most correctly termed paradoxical sleep.

► Sleep

Electrophilic

An electrophile or electrophilic compound is a reagent attracted to electrons that participates in a chemical reaction by accepting an electron pair in order to bond to a nucleophile. Because electrophiles accept electrons, they are Lewis acids. Most electrophiles are positively charged, have an atom which carries a partial positive charge, or have an atom which does not have an octet of electrons. The electrophiles attack the most electron-populated part of a ►**nucleophile**.

Electrospray Ionization Mass Spectrometry (ESI-MS)

Electrospray ionization mass spectrometry (ESI-MS) is an analytical method for mass determination of ionized molecules. It is a commonly used method for “soft” ionization of peptides and proteins in quadrupole, ion-trap, or time-of-flight mass spectrometers. The ionization is performed by application of a high voltage to a stream of liquid emitted from a capillary. The highly charged droplets are shrunk and the resulting peptide or protein ions are sampled and separated by the mass spectrometer.

- Proteomics
- Combinatorial Chemistry

Elimination Half-life

- Pharmacokinetics

Elimination of Drugs

The elimination of a drug is its removal from the body, either by chemical modification through metabolism or by removal from the body through the kidney, the gut, the lungs or the skin.

- Pharmacokinetics

EM-800

EM-800 is a pure estrogen receptor antagonist that has recently been used clinically for breast cancer patients who have failed tamoxifen therapy.

- Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
- Selective Sex Steroid Receptor Modulators

Embryonic Liver Fodrin (ELF)

A β -spectrin that plays a crucial role in mediation of TGF- β signaling through Smad proteins, and a potent tumor suppressor adaptor protein. Although different isoforms of ELF exist, ELF-3 is the longest at 2154 residues (8172 bases). ELF is composed of an actin-binding domain, a long repeat domain, and a short regulatory domain remarkable for the absence of a PH domain. Western blot analysis with a specific polyclonal antibody to ELF reveals a 200 kD protein is expressed ubiquitously. Utilizing embryonic stem cells, mouse knockouts of this gene have shown a phenotype with disrupted TGF- β signaling by Smad proteins. Homozygous *elf*^{-/-} mice demonstrate a phenotype similar to heterozygous *smad2*^{+/-} or *smad3*^{+/-} mice.

- Transforming Growth Factor-Beta

Emesis

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Synonyms

Vomiting

Definition

Emesis is the forceful involuntary expulsion of the stomach contents through the mouth. It is a reflex response that may be initiated by a number of stimuli.

Nausea is an unpleasant sensation of a desire to vomit or of an impending vomiting episode. When prolonged, it may occur in waves and may not always be followed by vomiting.

Retching is the process of emesis but without the actual expulsion of any of the stomach contents.

Basic Mechanisms

The vomiting reflex is controlled by the vomiting centre, a diffuse area in the medullary region of the brainstem. There are a number of ways to initiate the reflex (Fig. 1), including stimulation of sensory receptors in the alimentary canal, activation of the chemoreceptor trigger zone (CTZ) in the ▶area postrema, and excessive motion or other disturbances of the ▶labyrinth. Pregnancy, exposure to radiation, psychological or visual stimuli and various disease states such as migraine, diabetes or uraemia may also cause vomiting.

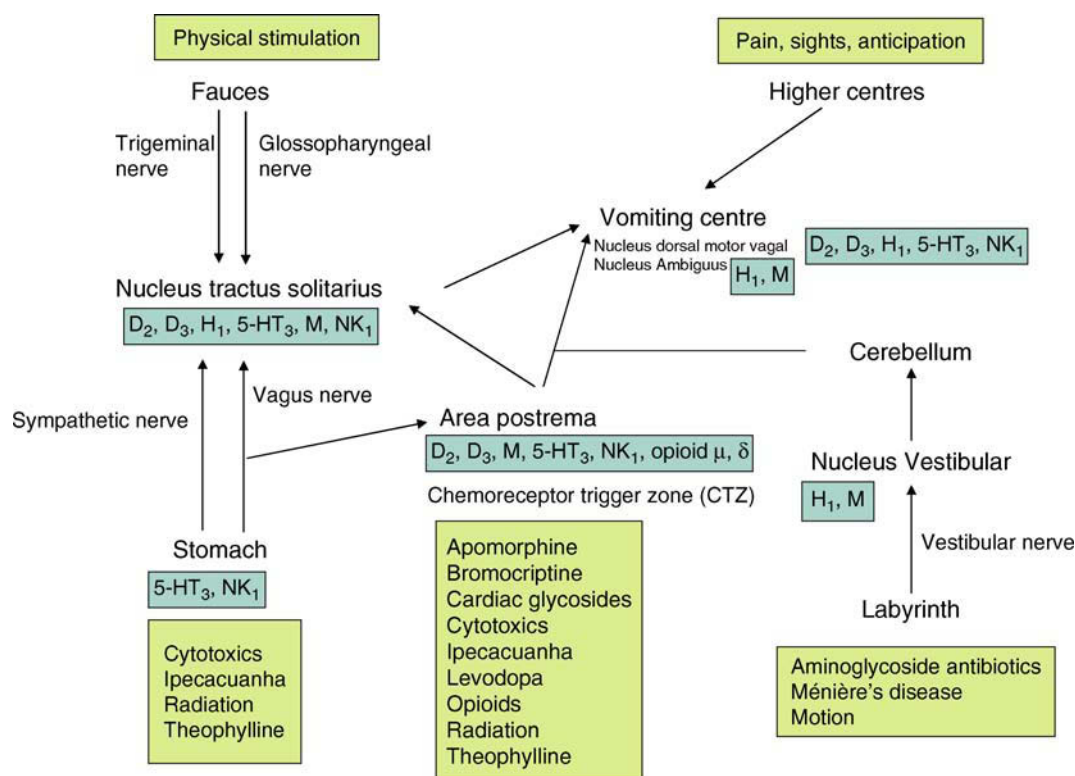
In the gastrointestinal tract, drugs or toxins, as well as mechanical stimulation, induce emesis by activation of sensory receptors on afferent neurons in the vagus and sympathetic nerves. Information is relayed to the vomiting centre via the nucleus tractus solitarius

(NTS) in the medulla. The area postrema lacks a ▶blood–brain barrier and is accessible to emetogens in the bloodstream. Neurons pass from the CTZ to the NTS and the vomiting centre.

Impulses from the vestibular apparatus in the labyrinth are conducted via the vestibular nucleus and cerebellum to the vomiting centre. Abnormal stimulation of the vestibular apparatus is involved in motion sickness and emesis, associated with ▶Ménière's disease.

Nausea and vomiting may also be induced by stimuli affecting higher centres of the brain, presumably the cortex, from unpleasant sights and smells as well as pain. Anticipatory vomiting also may occur during cancer chemotherapy. The ▶act of vomiting involves contraction of the diaphragm and abdominal muscles, to increase intragastric pressure. Also, retroperistaltic contractions of the intestine move the contents back into the stomach. Relaxation of the oesophagus and the cardiac sphincter at its lower end then allows the stomach contents to be vomited. The role of the stomach is passive, as active contraction is inhibited preceding emesis.

Nausea is often assumed to be a low level stimulation of the vomiting reflex. However, vomiting occurs without nausea in intestinal obstruction and in space



Emesis. Figure 1 Afferent pathways involved in vomiting. Some stimuli for initiation of vomiting from the various locations are shown in the boxes. The presence of receptors at a particular location does not imply that they are necessarily involved in normal transmission of the vomiting reflex.

motion sickness. Elevated plasma levels of ►**vasopressin** as well as tachygastria may play a role in nausea development.

Several neurotransmitters and ►**autacoids** are involved in initiating and conducting the vomiting reflex, including ►**acetylcholine**, **dopamine**, **histamine**, **serotonin**, **substance P (SP)** and possibly ►**prostaglandins**. Receptors for the neurotransmitters are known to occur in many central areas associated with pathways for initiating emesis (Fig. 1). However, the presence of receptors may not be always indicative of their involvement. For example, muscarinic receptors in the area postrema are probably not involved in motion sickness since all muscarinic receptor antagonists that are active as antiemetics have a non-quaternary structure, suggesting that they must be capable of crossing the blood–brain barrier.

Dopamine D₂ receptors are involved in some emetic responses but studies have also suggested that activation of D₃ receptors in the area postrema may either produce vomiting or enhance that elicited by D₂ receptor activation.

Release of serotonin from enterochromaffin cells in the gastrointestinal tract with activation of serotonin 5-HT₃ receptors on vagal afferents has an important role in the initiation of vomiting by anticancer drugs, exposure to radiation and in postoperative vomiting after abdominal surgery and may cause the vomiting seen on oral ingestion of hyperosmolar solutions of sodium chloride or after intravenous injection of erythromycin.

The neurokinin, substance P (SP), may be involved as a sensory transmitter in afferent vagal nerves involved in the vomiting reflex. Both SP and its receptors (NK₁ receptors) have been detected in several areas of the brain associated with vomiting, including the AP, NTS and dorsal motor vagal nucleus. The neurokinin can activate neurons in the AP and NTS. SP is present also in sensory nerves in the gut as well as being co-localised with serotonin in some enterochromaffin cells.

Clinical Aspects

Drug-Induced Emesis

Emetogenic drugs may be of value in treating cases of acute poisoning but usually nausea and vomiting induced by a drug are unwanted effects occurring in addition to its therapeutic action.

Emetics for Poisoning – Ipecacuanha

The most widely used emetic is syrup of ipecac, containing the alkaloids, emetine and cephaeline. Emetine induces vomiting by activation of sensory neurons in the vagus and sympathetic nerves to the stomach and centrally in the medulla, possibly at the CTZ. The release of serotonin and SP may be involved as 5-HT₃ and NK₁ receptor antagonists prevent emesis induced

by ipecacuanha. The use of syrup of ipecac in the treatment of poisoning is declining as activated charcoal is equally or more effective with fewer complications.

Cytotoxics

The incidence of vomiting in cancer chemotherapy is variable; the highly emetogenic cisplatin affects >90% of patients but some with low emetogenic potential such as vincristine, affect <10%. The time course of emesis also varies; cyclophosphamide induces a single phase that persists for 24 h whereas cisplatin produces a biphasic pattern; an acute phase, peaking at 6 h and a less intense second phase, peaking at day 2–3. Patients on repeated courses of chemotherapy often develop anticipatory vomiting and nausea, commencing several hours before treatment is given, subsequent to the initial course. This appears to be due to associative learning together with psychological stress from the drug regimen.

Cytotoxics cause an elevation of dopamine levels in the area postrema in animal studies and may release prostaglandins and inhibit enzymes such as enkephalinases to allow increased levels of enkephalins to activate opioid receptors on dopaminergic nerves.

Cytotoxics also cause cellular damage and the release of serotonin and other mediators from enterochromaffin cells. There is conflicting evidence regarding whether 5-HT₃ receptors in the medulla are activated also during chemotherapy and contribute to production of emesis. Currently, the weight of evidence favours peripheral 5-HT₃ receptors, with minor involvement of central receptors.

Release of SP from neurons in the AP, NTS and dorsal vagal motor nucleus may play a role in vomiting induced by cytotoxics. Based on the relative effectiveness of selective antagonists of 5-HT₃ receptors and NK₁ receptors against acute and delayed phases of cisplatin-induced vomiting, it has been suggested that serotonin has a greater role in the acute phase whereas SP has the major role in the delayed phase.

Dopamine Receptor Agonists

Parkinsonian patients receiving the dopamine precursor, levodopa or dopamine receptor agonists, such as bromocriptine and apomorphine may experience nausea and vomiting due to stimulation of dopamine D₂ receptors in the CTZ.

Opioids

Opioids act on the area postrema and/or the NTS via μ or δ receptors to produce emesis. Also, ambulatory patients receiving opioids are more affected than those confined to bed, suggesting a vestibular component in the effect. The emetic action of opioids is complicated by an antiemetic action, possibly involving two receptors at the NTS or the vomiting centre.

Morning Sickness and Hyperemesis Gravidarum

Morning sickness is experienced by many expectant mothers, during the first 3 months of gestation. Symptoms usually consist of nausea and retching rather than vomiting but a few women experience protracted vomiting (hyperemesis gravidarum). The basis for morning sickness is not known but has been variously considered to involve psychosomatic, endocrine, allergic and metabolic aspects. It may be a protective mechanism against ingestion of toxins or harmful chemicals in foodstuffs. Endocrine studies show hyperemesis gravidarum is associated with elevated serum levels of human chorionic gonadotrophin.

Motion Sickness

Motion sickness arises in the vestibular apparatus. Stimulation of the semicircular canals or the utricles by unfamiliar accelerating movement may cause a mismatch between the sensory information reaching the brain centres controlling balance and posture, with that anticipated. Motion sickness may be avoided by reducing 'sensory conflict'; fixing vision on a stable reference point, such as the horizon may be effective. Cortical centres may also contribute; memories of previous travel or the sight, and sounds of others being affected often increases susceptibility.

Postoperative Vomiting

The incidence of postoperative nausea and vomiting is variable, depending on a number of factors. These include the type of operations as well as the sex and age of the patient; women and children being more prone. Also, the type of anaesthetic and whether other drugs are used; opioids for example increase the incidence. Intraabdominal operations, neurosurgery and breast, eye, ear, nose or throat surgery have a high likelihood of nausea and vomiting. Overall incidence has been estimated at ca. 20–30%.

Pharmacological Intervention

Cannabinoids

Dronabinol (tetrahydrocannabinol), the active principle from cannabis and synthetic cannabinoids, nabilone and levonantradol are effective in treating nausea and vomiting in cancer chemotherapy. The mode of action is unclear but appears to involve cannabinoid CB₁ receptors. Cannabinoids have been shown to reduce acetylcholine release in the cortex and hippocampus, and have been suggested to inhibit medullary activity by a cortical action. Inhibition of prostaglandin synthesis and release of endorphins may also be involved in the antiemetic effect. A review of trials of dronabinol, nabilone or levonantradol concluded that while the cannabinoids were superior to placebo or dopamine receptor antagonists in controlling emesis

due to moderate emetogenic cancer chemotherapy, they produced harmful adverse effects more frequently.

Corticosteroids

Dexamethasone and methylprednisolone are useful antiemetics in chemotherapy, radiotherapy and postoperative vomiting, possibly acting by inhibiting central prostaglandin formation. They are used either alone for low emetogenic risk chemotherapy or in combination with 5-HT₃ receptor antagonists and aprepitant to improve control for moderate to highly emetogenic chemotherapy. Steroids have been found useful in the delayed, as well as acute phase of cisplatin-induced emesis. They can also be combined with metoclopramide and other dopamine receptor antagonists to enhance control of cytotoxic-induced vomiting. Used alone before induction of anaesthesia or in combination with 5-HT₃ receptor antagonists or high dose metoclopramide has improved control of postoperative vomiting.

Dopamine D₂-Like Receptor Antagonists

Several D₂-like receptor antagonists are used as antiemetics. They include phenothiazines (metopimazine, perphenazine, prochlorperazine, thiethylperazine), butyrophenones (droperidol, haloperidol), metoclopramide and domperidone. Metoclopramide also raises tone in the lower oesophagus and increases gastrointestinal motility and in high concentrations causes blockade of serotonin 5-HT₃ receptors. Domperidone does not readily cross the blood–brain barrier. It is particularly useful in controlling emesis associated with levodopa and dopamine receptor agonists used in Parkinsonism as it will not affect their beneficial action in the basal ganglia. The D₂-like receptor antagonists are useful in emesis occurring postoperatively and for cancer chemotherapy or radiotherapy but are not effective in motion sickness.

Metoclopramide, used in high doses, is more effective than other D₂ receptor antagonists in emesis due to cancer chemotherapy but its usefulness is limited by ▶**extrapyramidal effects**. Early clinical studies suggested variable effectiveness in postoperative nausea and vomiting but a recent multicentre-study has shown that a high dose administered intraoperatively, in combination with dexamethasone, produced effective control with minimal extrapyramidal effects. Metoclopramide is also effective in vomiting associated with migraine or uraemia and both metoclopramide and domperidone reduce nausea and vomiting associated with diabetic gastroparesis.

Histamine H₁ Receptor Antagonists (Histamine and Emesis)

Several histamine H₁ receptor antagonists are effective in treating motion sickness, Ménière's disease, morning sickness, uraemia and postoperative vomiting. They are not effective against cytotoxics. Antagonists with

piperazine-based structures (chlorcyclizine, cinnarazine, cyclizine, meclozine) or ethanolamine-based (dimenhydrinate, diphenhydramine, doxylamine) as well as promethazine are effective and appear to depend on central inhibition of histamine H₁ receptors and possibly also on an ability to inhibit muscarinic receptors. The non-sedating H₁ receptor antagonist, astemizole, is not effective in motion sickness. There has been considerable controversy regarding whether H₁ receptor antagonists pose a teratogenic risk when used to treat morning sickness but a meta-analysis has concluded that they can be used safely in pregnancy if nausea and retching cannot be controlled adequately by dietary modification.

Lorazepam

The benzodiazepine, lorazepam, acts allosterically on GABA_A receptors to facilitate the actions of GABA. Lorazepam has some antiemetic activity in cancer chemotherapy. When used in combination therapy, it does not appear to add to antiemetic control but may contribute to a reduction in anxiety.

Muscarinic Receptor Antagonists

These include atropine, scopolamine (hyoscine), trihexyphenidyl (benzhexol) and benztropine. They block central muscarinic receptors involved in various afferent pathways of the vomiting reflex (Fig. 1). They have been used to control motion sickness, emesis in Ménière's disease and postoperative vomiting. Currently, hyoscine is largely restricted to the treatment of motion sickness where it has a fast onset of action but a short duration (4–6 h). Administration of hyoscine by transdermal patch produces a prolonged, low-level release of the drug with minimal side effects. To control postoperative vomiting, it should be applied >8 h before emesis is anticipated.

Neurokinin NK₁ Receptor Antagonists

Aprepitant [MK-869, L-754030] is the only member of this class currently licensed for general clinical use. Animal studies have shown that several non-peptide NK₁ receptor antagonists inhibit emesis produced by the neurokinin, substance P and other emetogens such as apomorphine, morphine, ipecacuanha, cytotoxics and radiation. Clinical trials with non-peptide antagonists have relieved nausea and vomiting after gynaecological surgery and after cisplatin, and other chemotherapy. There is international consensus that combination of aprepitant with a 5-HT₃ receptor antagonist and dexamethasone produces the most effective control of vomiting after cyclophosphamide plus an anthracycline, as well as both acute and delayed emesis after cisplatin. Effectiveness of the combination against cisplatin is maintained over at least six cycles of therapy.

Aprepitant, which inhibits the cytochrome P450 isoform, CYP3A4, is metabolised by it. As a result,

aprepitant inhibits the metabolism of dexamethasone and methylprednisolone.

Pyridoxine

► **Pyridoxine** is used in morning sickness. Its mechanism of action remains unclear and several reviews of the use of pyridoxine have failed to find conclusive evidence of effectiveness. A recent double blind trial found pyridoxine to be of benefit in reducing nausea only. Some clinicians use pyridoxine as the first drug on the basis that it is the least likely to be toxic to the foetus.

Serotonin 5-HT₃ Receptor Antagonists

Those developed for clinical use as antiemetics include dolasetron, granisetron, ondansetron, palonosetron and tropisetron. They are effective against vomiting occurring postoperatively and that induced by cytotoxics or radiation. There is international consensus that these compounds are used first if vomiting occurs postoperatively in patients with no preoperative prophylaxis and that they are the preferred treatment in combination with dexamethasone for prevention of acute emesis induced by chemotherapy of moderate risk. For low to moderate risk radiation-induced emesis, there is consensus that they are the preferred antiemetic and for high risk, that they should be used in combination with dexamethasone. Preliminary evidence suggests they may also limit binge-vomiting in bulimics and ondansetron has been shown to reduce nausea, following intraduodenal infusion of lipids. The 5-HT₃ receptor antagonists are ineffective against motion sickness.

► Dopamine System

► Histaminergic system

► Muscarinic Receptors

► Serotonergic System

► Tachykinins and their Receptors

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Emphysema

A condition of the lung characterized by abnormal permanent enlargement of the air spaces distal to the terminal bronchioles accompanied by destruction of their walls and without obvious fibrosis.

► COPD

ENaC

► Epithelial Na⁺ Channel

N-End Rule

The N-end rule relates the in vivo half-life of a protein to the identity of its N-terminal residue. Proteins with destabilizing N-terminal residues such as arginine and leucine are recognized by a RING-type ubiquitin ligase (termed N-recognin or E3- α) that, together with a specific ubiquitin c, mediates poly-ubiquitylation.

► Ubiquitin/Proteasome System

Endocannabinoids

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Synonyms

Endogenous cannabinoids; Endogenous agonists of cannabinoid receptors

Definition

Endocannabinoids are endogenous mediators acting via the binding to, and activation of, ►cannabinoid receptors, CB₁ and CB₂ [1]. *N*-arachidonoyl-ethanolamine (AEA, anandamide) and 2-arachidonoyl-glycerol (2-AG) (Fig. 1) are the two most studied endocannabinoids. In the nervous system, endocannabinoids act as

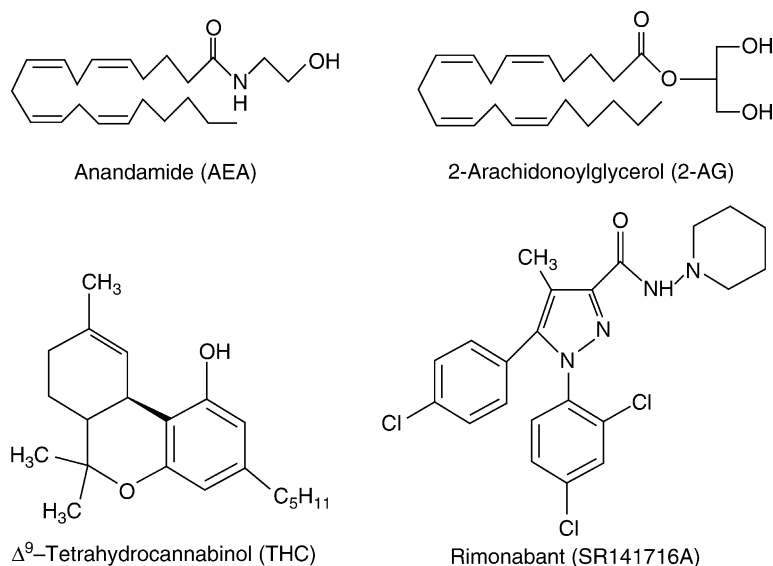
►neuromodulators. In peripheral non-nervous tissues, they act as autocrine or paracrine regulators of the homeostasis of other chemical mediators.

Basic Characteristics

Endocannabinoid Biosynthesis

Both AEA and 2-AG are biosynthesized “on demand” and released from cells immediately after their production. These events are triggered by the enhancement of intracellular Ca²⁺ concentrations that follows cell depolarization or the mobilization of intracellular Ca²⁺ stores subsequent to stimulation of G_{q/11} protein-coupled receptors [2]. However, in many cases, the levels of the two main endocannabinoids are modulated in different and sometimes even opposing ways via the modulation of the activity and/or expression of either biosynthetic or degrading enzymes or both. AEA is produced from the processing of *N*-arachidonoyl-phosphatidylethanolamine (NArPE), which in turn is obtained from the enzymatic transfer of arachidonic acid esterified on the *sn*-1 position of phospholipids to the nitrogen atom of phosphatidylethanolamine, via an as-yet unidentified *N*-acyltransferase. The enzyme catalyzing the one-step conversion of NArPEs and other *N*-acylphosphatidylethanolamines (NAPEs) into AEA and other *N*-acyl-ethanolamines is the same and is termed NAPE-selective phospholipase D (NAPE-PLD). It is: (i) enzymatically distinct from other PLD enzymes, (ii) stimulated by Ca²⁺, and (iii) a member of the β -lactamase fold of the zinc-metallo-hydrolase family of enzymes. Its over-expression in cells leads to higher cellular levels of AEA and correspondingly lower levels of NArPE, thus supporting its role in the biosynthesis of this endocannabinoid. However, three more pathways have been identified that can transform NArPE into AEA. In macrophages, a phospholipase C (PLC) converts NArPE into AEA via phospho-AEA. PTPN22, a protein tyrosine phosphatase, is the enzyme catalyzing phospho-AEA hydrolysis to AEA. In brain homogenates, NArPE can be converted into 2-lyso-NArPE, via the action of a group IB soluble phospholipase A₂, or into glycerophospho-AEA, via alpha/beta-hydrolase 4. AEA is then formed via the hydrolysis of 2-lyso-NArPE by a selective lysophospholipase D, or of glycerophospho-AEA by a phosphodiesterase, respectively (Fig. 2).

2-Arachidonate-containing DAGs are the most frequent biosynthetic precursors of 2-AG. They are produced from the hydrolysis of phosphoinositol-bis-phosphate (PIP₂), catalysed by the PIP₂-selective PLC, or, in some cases, from the hydrolysis of phosphatidic acid, catalysed by a phosphohydrolase. DAGs are then converted into 2-AG by the action of two plasma membrane *sn*-1-selective DAG lipases, DAGL α and DAGL β (Fig. 2). These two enzymes: (i) contain the typical lipase-3 and Ser-lipase signature sequences, in which two highly conserved amino acid residues, Ser443 and Asp495, are



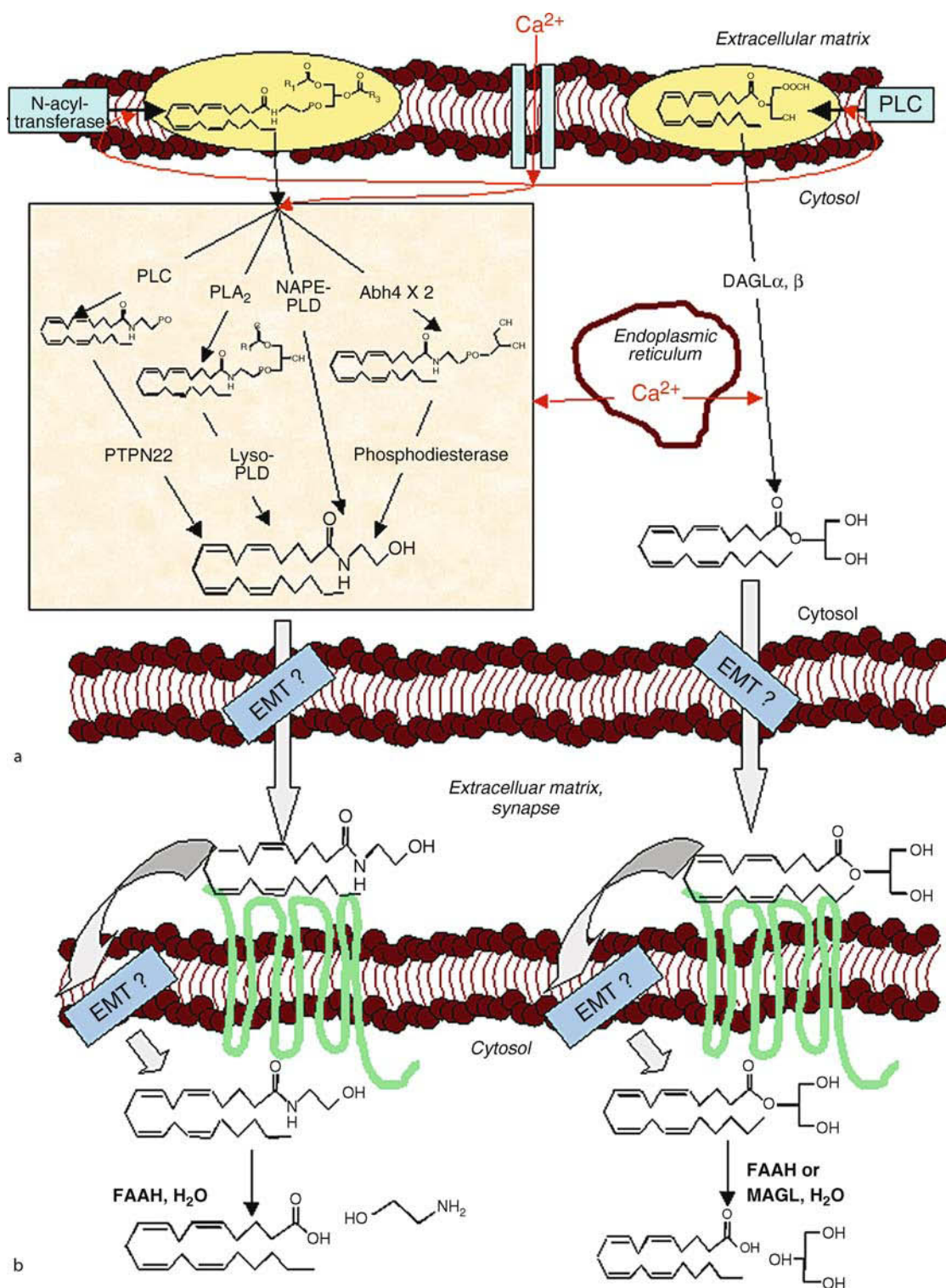
Endocannabinoids. Figure 1 Chemical structures of the two most studied endocannabinoids, anandamide and 2-arachidonoylglycerol, of *Cannabis sativa* psychoactive principle, Δ⁹-tetrahydrocannabinol, and of the CB₁ receptor antagonist/inverse agonist, rimonabant.

necessary for enzymatic activity, (ii) exhibit also four hydrophobic, possibly *trans*-membrane, domains near their N terminus, (iii) are stimulated by Ca²⁺ and (iv) do not prefer DAGs with any particular fatty acyl chain in the *sn*-2 or *sn*-1 position, although they exhibit strong selectivity for DAGs over phospholipids, monoacylglycerides, triacylglycerols and fatty acid amides. DAGLα is more abundant in the adult brain, and DAGLβ in the developing brain. Both enzymes are co-localized with CB₁ receptors in neuronal axons of the perinatal nervous system, and “move” to post-synaptic neurons in the adult brain. DAGLα is found in post-synaptic dendritic spines establishing synapses with CB₁ expressing axons, thus supporting the proposed roles for 2-AG as a ►retrograde messenger of ►synaptic plasticity in the adult brain.

Endocannabinoid Action

Once released from cells, endocannabinoids bind to cannabinoid receptors and elicit typical CB₁- and CB₂-mediated intracellular signalling events, thereby leading to biological responses typical of cannabinoid receptor agonists [1, 2]. However, the functional role of endocannabinoids cannot be inferred only from the knowledge of the pharmacological effects of Δ⁹-►tetrahydrocannabinol (THC) and synthetic cannabinoids for several reasons: (i) because of the mechanisms underlying their biosynthesis and because of their rapid metabolism, endocannabinoids appear to be produced only “when and where” needed. Thus, for example, if endocannabinoids are being made and released to protect a neuron from glutamate-induced excitotoxicity, they will be released only from the post-synaptic neuron undergoing excessive stimulation

by glutamate, to inhibit only glutamate release from the pre-synaptic glutamatergic neuron, without inhibiting the activity of GABA-ergic interneurons, which, by releasing GABA, also tone down excitotoxicity. This specificity of endocannabinoid action is not always maintained when CB₁ receptors are activated by exogenous agonists, (ii) in some cases endocannabinoids are biosynthesized together with congeners that are inactive per se at cannabinoid receptors, but can nevertheless modulate the activity of AEA and 2-AG or even act at different receptors, (iii) endocannabinoids, and AEA in particular, can act also at non-cannabinoid receptors, including ion channels and receptors for neurotransmitters or other neuromodulators and (iv) arachidonic acid produced from the degradation of exogenously administered endocannabinoids (see below) can be used to generate other mediators, which in turn may act on other targets. Therefore, to understand endocannabinoid action, pharmacological studies using cannabinoid CB₁ and CB₂ receptor agonists and ►antagonists/inverse agonists, and inhibitors of endocannabinoid metabolism, must be accompanied by observations on the phenotype of CB₁, CB₂ and FAAH “knock-out” mice, and by the quantification of anandamide and 2-AG levels in various tissues under physiological and pathological conditions (see below). It was established that, in the brain, endocannabinoids act as retrograde messengers acting on pre-synaptic CB₁ receptors in both short-term and long-term forms of synaptic plasticity. These retrograde actions underlie at least part of CB₁-mediated regulation of cognitive and emotional functions in the brain, reinforcement of substances of abuse in the mesolimbic system,



Endocannabinoids. Figure 2 Proteins and pathways for the biosynthesis and degradation of endocannabinoids. Abbreviations used: 2-AG, 2-arachidonoyl glycerol; Abh4, alpha/beta-hydrolase 4; FAAH, fatty acid amide hydrolase; DAGL, *sn*-1-selective diacylglycerol lipase; EMT, putative endocannabinoid membrane transporter; MAGLs, monoacylglycerol lipases; NAPE-PLD, *N*-acylphosphatidyl-ethanolamine-selective phospholipase D; lyso-PLD, lysophospholipase D; PLA₂, phospholipase A₂; PLC, phospholipase C; PTPN22, protein tyrosine phosphatase N22. Arrows denote activation, transport or transformation. Adapted from Matias et al. (2007) *Br. J. Pharmacol.*, 152, 667–690.

induction of appetite or reduction of satiety in the hypothalamus and brainstem, and control of movement and posture in the basal ganglia and cerebellum. Neuromodulatory, CB₁-mediated actions of endocannabinoids in the sensory and autonomic nervous systems result in the regulation of circulatory and gastrointestinal functions and the hypothalamic-pituitary-adrenal axis. Direct autocrine or paracrine actions in non-neuronal cells underlie the several CB₁- and CB₂-mediated effects of these compounds on female and male reproduction, bone formation, immune response and adipocyte, and β -cell function.

Endocannabinoid Inactivation

AEA is inactivated through intracellular enzymatic hydrolysis to arachidonic acid and ethanolamine (Fig. 2). The enzyme catalyzing this reaction was named “fatty acid amide hydrolase” (FAAH) as it recognizes as substrates also other long chain fatty acid amides, including *N*-acylethanolamines, primary amides, *N*-acyl-aminoacids and *N*-acyl-taurines. FAAH catalyses efficiently also the hydrolysis of long chain fatty acid esters, including 2-AG. The physiopathological role of FAAH as a major endocannabinoid-inactivating enzyme has been assessed through: (i) the study of the phenotype of transgenic mice lacking the enzyme, i.e. the “FAAH knockout mice”, (ii) the design and pharmacological testing in vivo of specific FAAH inhibitors, (iii) immunohistochemical studies describing the tissue and cellular distribution of the enzyme and its relationship with cannabinoid receptor distribution and (iv) the identification of *Faah* gene polymorphisms associated with disorders such as obesity, propensity to drug addiction and schizophrenia.

In addition, Monoacylglycerol lipase (MAGL) enzymatic activities inactivate 2-AG (Fig. 2). A MAGL was cloned recently from the rat and evidence for its role in 2-AG degradation in isolated cells was provided by the use of “silencing RNA” techniques. The cloned MAGL seems to account for only 50% of the total 2-AG-hydrolyzing activity in soluble fractions of rat brain, and pharmacological evidence for the existence of other MAGL isoforms has been provided. The cloned MAGL recognizes as substrates both *sn*-1 and -2-acylglycerols with almost any unsaturated long chain fatty acid esterified to the glycerol backbone, but is inactive with fatty acid amides. It is distributed in the CNS in the same brain regions as CB₁ receptors and is a pre-synaptic enzyme, in agreement with the necessity of inactivating 2-AG acting as a retrograde signal. However, our current knowledge of the role of MAGL is still limited due to the lack of a “knockout” mouse for this enzyme and of selective inhibitors. Whether FAAH degrades 2-AG constitutively is still controversial. Selective FAAH inhibitor does produce a significant

elevation of 2-AG tissue levels under certain conditions of administration and in certain tissues.

In cell free systems or isolated cells, some enzymes of the arachidonate cascade can also recognize AEA and 2-AG as substrates, thereby producing the corresponding lipoxygenase and cyclooxygenase-2 derivatives. However, these metabolites have not yet been isolated from tissues and their biological relevance is still unknown.

Endocannabinoid Membrane Transport

AEA and 2-AG need to be transported across the membrane in order to interact with either cannabinoid receptors or intracellular hydrolyzing enzymes (Fig. 2) [3]. It is still being debated whether or not endocannabinoid uptake by, and release from, cells occurs via a membrane transporter, and not via simple **passive diffusion**. Intracellular degradation of AEA by FAAH was suggested to be the only drive behind its cellular uptake. However, strong indirect evidence exists for specific proteins facilitating the membrane transport of both AEA and 2-AG. Experiments carried out using cells from FAAH knockout mice, confocal microscopy to assess the spatial and functional separation between anandamide uptake and hydrolysis, and synthetic inhibitors capable to distinguish between FAAH and proteins responsible for the cell membrane binding/cellular uptake of AEA, favour the existence of one or more specific proteins for AEA transport. Nevertheless, no such protein has been cloned or identified to date.

Drugs

Pathological Conditions in Which Endocannabinoids are Involved

Endocannabinoid levels often undergo dramatic tissue-specific changes in both animal models of disorders and in human diseases (Table 1) [4]. During some acute pathological states or transient near-physiological perturbations of the normal homeostasis of the organism, the levels of at least one endocannabinoid are elevated only in the tissues specifically involved in the disorder to help re-establishing the levels of other endogenous mediators. This occurs, for example, following insults or stressful stimuli ranging from brief food deprivation, restriction-induced stress, and retrieving of aversive memories to administration of acute painful stimuli, head injury, and ischemia. In the case of progressive/chronic disorders, endocannabinoid levels can become permanently elevated, although again in a tissue-selective way. This might lead again to homeostatic (e.g. anti-inflammatory or cell protective) effects in ways mediated by CB₁- or CB₂ receptors (or also by non-cannabinoid receptors), as in the case of some types of cancer (breast and colorectal carcinoma, glioma, etc.) or inflammatory gastrointestinal disorders (colitis,

Endocannabinoids. Table 1 Changes of endocannabinoid levels during pathological conditions and subsequent possible pharmacological interventions

Disorder	Effects on endocannabinoid levels	Potential drugs
<i>Neurodegenerative/neuromotor disorders:</i>		
1. Parkinson's disease (PD)	1. In a non-human primate model of PD endocannabinoid levels are elevated in the basal ganglia and may contribute to the generation of parkinsonian symptoms and/or to expression of levodopa-induced dyskinesia. The cerebrospinal fluid of untreated PD patients contains elevated levels of AEA	1. CB ₁ antagonists or biosynthesis inhibitors
2. Alzheimer's disease (AD)	2. In the hippocampus of β-amyloid-treated rats, an animal model of AD, 2-AG levels are elevated and exert neuroprotection but also participate in memory retention loss	2. Inhibitors of cellular re-uptake or CB ₁ antagonists, possibly depending on the phase of the disorder
3. Amyotrophic lateral sclerosis (ALS)	3. AEA and 2-AG increase in the spinal cord of SOD1 transgenic mice, a model of ALS, to inhibit disease progress	3. CB ₂ receptor agonists or inhibitors of degradation
4. Multiple sclerosis (MS)	4. In rats with EAE, an animal model of multiple sclerosis, AEA and 2-AG levels are decreased in the striatum and midbrain. This might be associated with motor impairment	4. Inhibitors of degradation (both FAAH and cellular re-uptake)
<i>Neuronal excitotoxicity</i>	AEA levels are elevated in the hippocampus of mice treated with kainic acid. 2-AG levels are elevated in rats treated with pilocarpine These are two animal models of epileptic seizures, where the endocannabinoids play an anti-convulsant and protective function	Inhibitors of cellular re-uptake
<i>Neuropathic pain</i>	Endocannabinoid levels are elevated in periaqueductal grey, rostral ventral medulla and spinal cord during chronic constriction injury of the sciatic nerve in the rat, probably to inhibit pain	• FAAH inhibitors
		• Cannabis extracts (e.g. Sativex)
<i>Gastrointestinal disorders:</i>		
1. Colon inflammation	1. AEA levels are elevated in the colon of DNBS-treated mice and in the colon submucosa of TNBS-treated rats, two animal models of inflammatory bowel diseases, and in the biopsies of patients with ulcerative colitis, to control inflammation	1. Inhibitors of degradation (both FAAH and cellular re-uptake)
2. Cholera toxin-induced diarrhoea	2. Increased AEA levels after administration of cholera toxin to mice, a model of diarrhoea, exert anti-secretory action in the small intestine	2. Inhibitors of cellular reuptake
3. Diverticular disease	3. Increased AEA levels in colon strips from patients with diverticular disease participate in alterations of neural control of colon motility	3. None tested
4. Paralytic ileus	4. Increased AEA levels participate in inhibition of small intestine motility	4. CB ₁ antagonists
<i>Eating and metabolic disorders:</i>		
1. Anorexia nervosa (AN) and Binge-eating disorder (BED)	1. Increased blood levels of AEA in patients with AN and BED may participate in reward aspects of aberrant eating behaviours	1. None tested

Endocannabinoids. Table 1 Changes of endocannabinoid levels during pathological conditions and subsequent possible pharmacological interventions (Continued)

Disorder	Effects on endocannabinoid levels	Potential drugs
2. Obesity and hyperglycemia	2. 2-AG levels are elevated in mouse adipocytes and epididymal of mice with DIO. AEA and 2-AG levels are elevated in rat insulinoma β -cells, in pancreas of mice with DIO, and in obese women. Patients with obesity or hyperglycaemia caused by type 2 diabetes exhibit elevated levels of 2-AG or of both endocannabinoids in visceral fat or blood, respectively. AEA levels are elevated in the liver of DIO mice	2. CB ₁ antagonists
Cancer	Elevated levels of AEA in glioblastomas, increased levels of 2-AG in meningiomas, elevated levels of both AEA and 2-AG in colorectal carcinoma, with possible anti-tumour action	Inhibitors of degradation (both FAAH and cellular re-uptake)

Recent data are shown on those pathological conditions in which endocannabinoid levels are found to be altered in the tissues or organs affected by the disorder. Depending on the possible functional significance of these changes, pharmacological intervention with degradation inhibitors/agonists or with biosynthesis inhibitors/antagonists is suggested as a possible therapeutic strategy

AEA, anandamide; 2-AG, 2-arachidonoyl glycerol; EAE, experimental autoimmune encephalomyelitis; DIO, diet-induced obesity

diverticular disease, celiac disease) [4, 5]. However, a chronic over-activity of CB₁ receptors might eventually contribute to the development of also some of the symptoms typical of the disorder. This situation has been described for some experimental models of Parkinson's disease (PD) and Alzheimer's disease (AD), of genetic and diet-induced obesity, and of hepatic fibrosis, osteoporosis, ►paralytic ileus and ►noxious hypotension [3, 5]. In the female reproductive system, a critical balance between anandamide synthesis and degradation in mouse embryos and oviducts creates the appropriate conditions for normal development of embryos and their oviductal transport, the malfunctioning of which possibly explains the association observed between lower FAAH/higher AEA levels in the blood of pregnant women and pre-term abortion. Finally, a reduction of endocannabinoid levels is found in the brain of mice with experimental allergic encephalomyelitis, a mouse model of multiple sclerosis, in animal models of Huntington's chorea, and in patients with migraine. The subsequent impaired CB₁ signalling might explain some of the clinical hallmarks of these disorders (Table 1).

Endocannabinoid-Based Drugs

Based on the role of endocannabinoids and cannabinoid receptors in several pathological conditions, the pharmacological manipulation of their levels or action is being developed as a therapeutic strategy. Enhancement of endocannabinoid signalling when this plays uniquely a protective role can be effected in a safer way using: (i) cannabis extracts in which the presence of non-psychoactive cannabinoids with therapeutic activity per

se mitigates the unwanted CB₁-mediated effects of THC and potentiates those of more therapeutic value, (ii) synthetic compounds specific for CB₂ receptors and (iii) inhibitors of endocannabinoid degradation, which are likely to act only in those tissues where there is an ongoing turnover of endocannabinoids. Inhibitors of endocannabinoid action or biosynthesis will be of benefit, instead, in those conditions where endocannabinoids contribute to the symptoms or progress of disease. The marketing in Europe of a CB₁ receptor antagonist/inverse agonist (rimonabant, Acomplia®) (Fig. 1) against obesity and the ►metabolic syndrome, and in Canada of Sativex®, a cannabis extract, against neuropathic pain in multiple sclerosis, demonstrate that it is possible to interact safely with the endocannabinoid system, and open the way to future studies in disorders ranging from neuronal damage, anxiety and depression to chronic pain, hypertension, cancer and gastrointestinal disorders [4, 5].

- Camabis
- Appetite Control
- Anti-obesity Drugs
- Emesis
- Metabolic Syndrome

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Endocytosis

A process in which a substance gains entry into a cell. Endocytic mechanisms are crucial for a variety of cellular functions such as the uptake of nutrients, regulation of cell surface expression of receptors, maintenance of cell polarity, and more. Receptor-mediated endocytosis via clathrin-coated pits is the most studied endocytic process, which is important for regulation of the time and magnitude of signals generated by a variety of cell-surface receptors.

- Intracellular Transport
- Low-density Lipoprotein Receptor Gene Family
- Growth Factors

Endogenous Antipyresis

The inherent mechanism that prevents the height of fever from reaching a potentially dangerous level. It is mediated by substances liberated both systemically and within the brain during fever that counter the formation or action of endogenous pyrogens, or inhibit the activity of neural circuits that modulate febrigenesis.

Endogenous antipyretics include certain cytokines, neuropeptides, glucocorticoids, hormones, nitric oxide, and others.

- Fever

Endogenous Opioid Peptides

Endogenous opioid peptide released both in the central nervous system and in other apparatuses of the body that have many regulatory functions, including inhibition of pain transmission.

Endogenous opioid peptides are the wide variety of endogenous peptides isolated since 1975 which are the natural ligands for the opioid receptors. The peptides

derive from three precursor molecules (proopiomelanocortin, proenkephalin, prodynorphin); each encoded by a separate gene. The discovery of the endomorphins, two amidated tetrapeptides which do not derive from the three precursor molecules, indicate the existence of additional opioid peptide genes which, however, have not yet been identified.

- Opioid Systems
- Analgesics
- Placebo Effect

Endometriosis

An estrogen dependent disorder characterized by the presence of uterine tissue at sites other than the uterus such as the peritoneum, ovaries and rectovaginal and rarely in the pericardium, pleura and brain.

- Aromatase
- Selective Sex Steroid Receptor Modulators
- Receptor Modulators

Endopeptidase

A peptidase that can cleave peptide bonds within a protein or peptide. Endopeptidases are classified in Enzyme Nomenclature according to catalytic type and are included in sub-subclasses 3.4.21–3.4.24.

- Non-viral Peptidases

Endoplasmic Reticulum (ER)

A system of membrane enclosed cisternae in the cytoplasm. The ER is continuous with the outer membrane of the nuclear envelope. The part of the ER coated with ribosomes is called rough ER, the other part is called smooth-surfaced ER. The rough ER is the first compartment of the secretory pathway. Here, membrane proteins are integrated into and secretory proteins translocated across the ER membrane. Furthermore,

protein folding is established and checked by a quality control system. From the ER, proteins are delivered in vesicles to the ERGIC.

- ▶ Protein Trafficking and Quality Control
- ▶ Intracellular Transport

Endorphins

Endorphins belong to the group of endogenous opioid peptides.

- ▶ Opioid System
- ▶ Analgesics

Endosome

Intracellular vesicle that carries complexes of endocytic receptors with their ligand cargo internalized from the cell surface.

- ▶ Endocytosis
- ▶ Intracellular Transport
- ▶ Low-density Lipoprotein Receptor Gene Family

Endothelial Cells

Endothelial cells are the cells in the inner layer of blood vessels.

- ▶ Endothelins
- ▶ Vascular Endothelial Growth Factor
- ▶ Nitric Oxide
- ▶ Purinergic System

Endothelial Lipase (EL)

Endothelial-anchored enzyme in multiple tissues primarily responsible for hydrolysis of phospholipids in HDL.

- ▶ Lipoprotein Metabolism

Endothelial Nitric Oxide Synthase (eNOS)

Synonyms

eNOS

Definition

A constitutive enzyme binding to caveolin-1 in the plasma membrane, mainly in endothelial cells. It is activated in the presence of Ca^{2+} and calmodulin.

- ▶ Nitric Oxide

Endothelin Converting Enzyme

Endothelin Converting Enzymes (ECEs) belong to the family of metalloproteases that catalyze the proteolytic activation of big endothelins.

- ▶ Endothelins
- ▶ Matrix Metalloproteases

Endothelins

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Definition

Endothelins comprise a family of three vasoactive isopeptides of 21 amino acids that have an essential role in the regulation of the vascular and bronchiolar tone and the control of natriuresis in the kidney. Endothelin peptides are also involved in nociception and have a critical role in the progression of prostate and ovarian cancer.

Basic Characteristics

Endothelin and Endothelin-converting Enzymes

In 1985, a peptide was described in the supernatants of endothelial cells that mediated vasoconstriction [1]. This peptide was isolated and sequenced, and the cDNA was cloned. According to its origin from endothelial cells it was named endothelin.

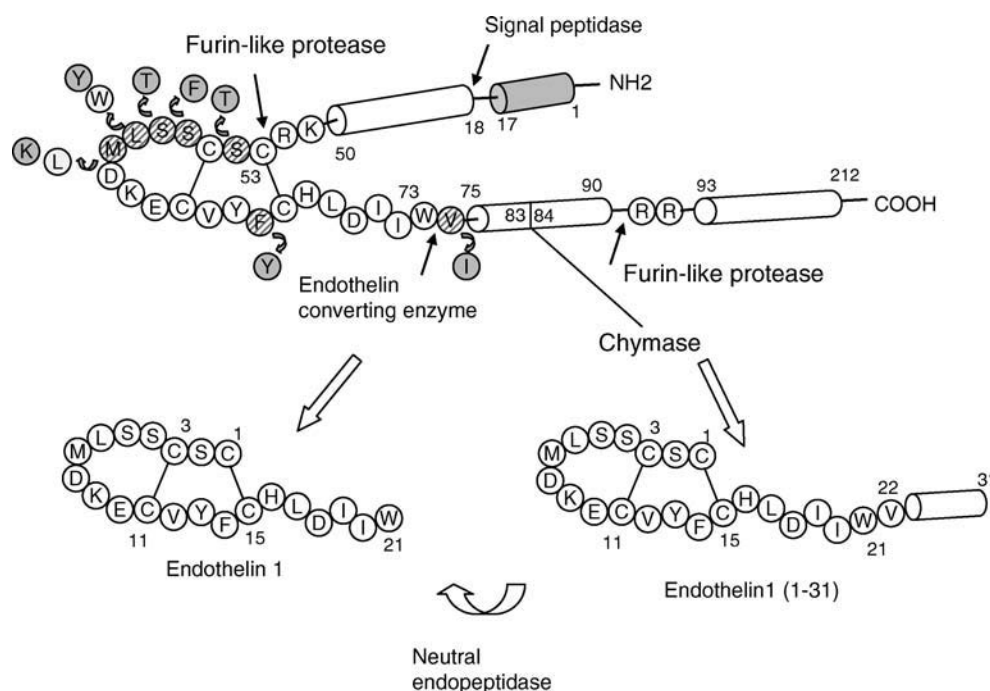
To date, three endothelin isoforms are known (ET-1, ET-2, ET-3), encoded by different genes [2]. Endothelins are synthesized as prepolypeptides of approximately 200 amino acids (Table 1). The biological active endothelins are generated in a two-step proteolytic process (Fig. 1). In the first step, ►**furin-like proteases** generate big-endothelins (big-ETs) of 38–41 amino acids that are biologically inactive. In a second step, specific ►**endothelin-converting enzymes** (ECEs) specifically cleave big-ETs between tryptophan 21 and valine/isoleucine 22, thereby producing the mature endothelins. Big-ET-1 was also found to be cleaved by mast cell ►**chymase** resulting in ET-1(1–31). Whilst ET-1(1–31) is not a substrate of ECEs, it is further processed by neutral

endopeptidase (NEP) to the biological active ET-1. ►**Matrix metalloproteinase 2** (gelatinase A) also cleaves big-ET-1 and yields ET-1(1–32). Whether ET-1(1–32) is also a substrate of NEP remains to be shown.

ECEs are ►**metalloproteinases** that are homologous to the ►**neutral endopeptidase** (NEP, E-24.11, neprilysin); unlike NEP, however, they form disulfide-bonded homodimers. In man, with ECE-1 and ECE-2, two isoforms are known, which are encoded by two separate genes. For ECE-1 four different variants have been identified (ECE-1a–d), which are generated by the use of alternative promoters (Table 2). The ECE-1 isoforms only differ in their N-terminal amino acid sequence. For ECE-2, a single gene product has been described in

Endothelins. Table 1 Human endothelin isoforms

	Number of amino acids in		Chromosome	Diseases
	Prepro-ET-1	Big-ET-1		
hET1	212	38	6p23–24	
hET2	178	38	1p34	
hET3	238	41	20q13.2–13.3	Hirschsprung's disease, Waardenberg syndrome, type 4



Endothelins. Figure 1 Processing of prepro-ET-1. ET-1 is generated as a preprohormone consisting of 212 amino acids. Amino acids 53–90 represent big-ET-1, amino acids 53–73 mature ET-1. Amino acids that vary between the three endothelin isoforms are depicted by hatched circles (only variant amino acids of the mature ET-1 moiety and of the ECE-cleavage site are indicated). The amino acids present in ET-2 and ET-3 are depicted by light or dark grey circles, respectively. The signal peptide (grey cylinder) is cleaved off in the endoplasmic reticulum by the signal peptidase. Big-ET-1 is generated by proteolytical processing (furin-like proteases), which recognize dibasic amino acids motifs. Mature ET-1 is formed after processing through the endothelin converting enzyme. Mast cell chymase generates ET-1(1–31), which is further processed by NEP to yield ET-1.

Endothelins. Table 2 Properties of the different isoforms of endothelin-converting enzymes

	Subcellular localization	pH Optimum	Amino acids (in human)	Species
ECE1a	Pm	6.8–7.2	758	Human, bovine, rat
ECE1b	i.c.	6.8–7.2	770	Human, bovine, rat
ECE1c	Pm late endosomes/multivesicular bodies	6.8–7.2	754	Human
ECE1d	i.c. recycling endosomes	6.8	767	Human, rat
ECE2a	i.c.	5.5	787	Human, mouse, bovine
ECE2b	?	?	765	Human, mouse, bovine
ECE3	?	6.6	-	Bovine

pm: plasma membrane, i.c.: intracellular.

man, whereas in mouse and calf two splice variants have been identified. In calf, an ECE-3 isoform has been isolated from iris microsomes and the choroid plexus, which is specifically involved in the conversion of big-ET-3 to ET-3. A human homologue of bovine ECE-3 has not yet been identified.

The ECE isoforms show different subcellular distributions and enzymatic characteristics (Table 2). ECE-1a and ECE-1c are mainly expressed at the cell surface, whereas ECE-1b, ECE-1d and ECE-2 are expressed intracellularly. Plasma membrane-bound ECE cleaves big-ET-1 circulating in the blood, whereas intracellular ECE isoforms are involved in the generation of mature endothelins. In addition, ECEs (as well as NEP and the insulin-degrading enzyme) contribute to the degradation of amyloid β (A β) peptide.

Human umbilical vein endothelial cells (HUVEC) express the isoforms ECE-1a, -1b, -1d and ECE-2. In these cells, ET-1 is secreted *via* both a constitutive and a regulated pathway. The ratio of released ET-1:big-ET-1 is 4:1. About 80% of the ET-1 is secreted at the abluminal cell surface of endothelial cells. ECE-isoforms are abundantly expressed on the cell surface of endothelial cells and to a lower level also on vascular smooth muscle cells. In atherosclerotic lesions of vessels, however, ECE expression in smooth muscle cells is upregulated. ECE isoforms expressed in smooth muscle cells contribute significantly to the generation of mature ET in normal and in particular atherosclerotic vessels.

Endothelial cells are the major source of ET-1-synthesis. ET-1 is also produced by astrocytes, neurons, hepatocytes, bronchial epithelial cells, renal epithelial and mesangial cells. Physiological stimuli of ET-1-synthesis in endothelial cells are angiotensin II, catecholamines, thrombin, growth factors, insulin, hypoxia and shear stress. Inhibitors of ET-1 synthesis are atrial natriuretic peptide, prostaglandin E2 and prostacyclin. ET-2 is mainly synthesized in kidney, intestine, myocardium and placenta and ET-3 is predominantly produced by neurons, astrocytes and renal epithelial cells.

Endothelin Receptors

Endothelins exert their diverse actions *via* two G protein-coupled receptors named endothelin A (ET_A) and endothelin B (ET_B) receptor, which share an identity of about 64% in their amino acid sequences. Both receptors display a signal peptide that is required for the correct biogenesis. After the N terminus is accessible in the ER lumen, the signal peptide is cleaved off (comprising 20 and 26 amino acids in ET_A and ET_B receptors, respectively). Further post-translational modifications are ►Asn-linked glycosylation of the extracellular N terminus (hET_A: Asn29, Asn 62; hET_B Asn 59) and ►palmitoylation of cysteine residues in the intracellular C terminus. Mass spectrometry of the bovine ET_B receptor revealed that cysteine residues 402 and 404 are palmitoylated. The palmitoylation of endothelin receptors is essential for the activation of G proteins, since palmitoylation-deficient ET_A and ET_B receptors fail to stimulate G_{q/11} and G_i proteins, respectively. Disulfide bonds between the highly conserved cysteine residues of the first (ET_A: Cys158, ET_B: Cys174) and the second extracellular loop (ET_A: Cys239, ET_B: Cys255) are also likely. Whether cysteine residues in the extracellular N terminus (ET_A: Cys69, ET_B: Cys90) and the third extracellular loop (ET_A: Cys341, ET_B: Cys359) also form disulfide bonds has not been clarified. The extracellular N terminus of the ET_B receptor undergoes agonist-induced limited proteolysis. Upon binding of ET-1 the N terminal 38 amino acids are cleaved off by a metalloprotease. The cleavage involves the single Asn-linked glycosylation site, resulting in a non-glycosylated receptor. The functional role for the N-terminal cleavage is not known, yet. However, genetic deletion of the N terminus or proteolytic removal results in an ET_B receptor with an altered signal transduction.

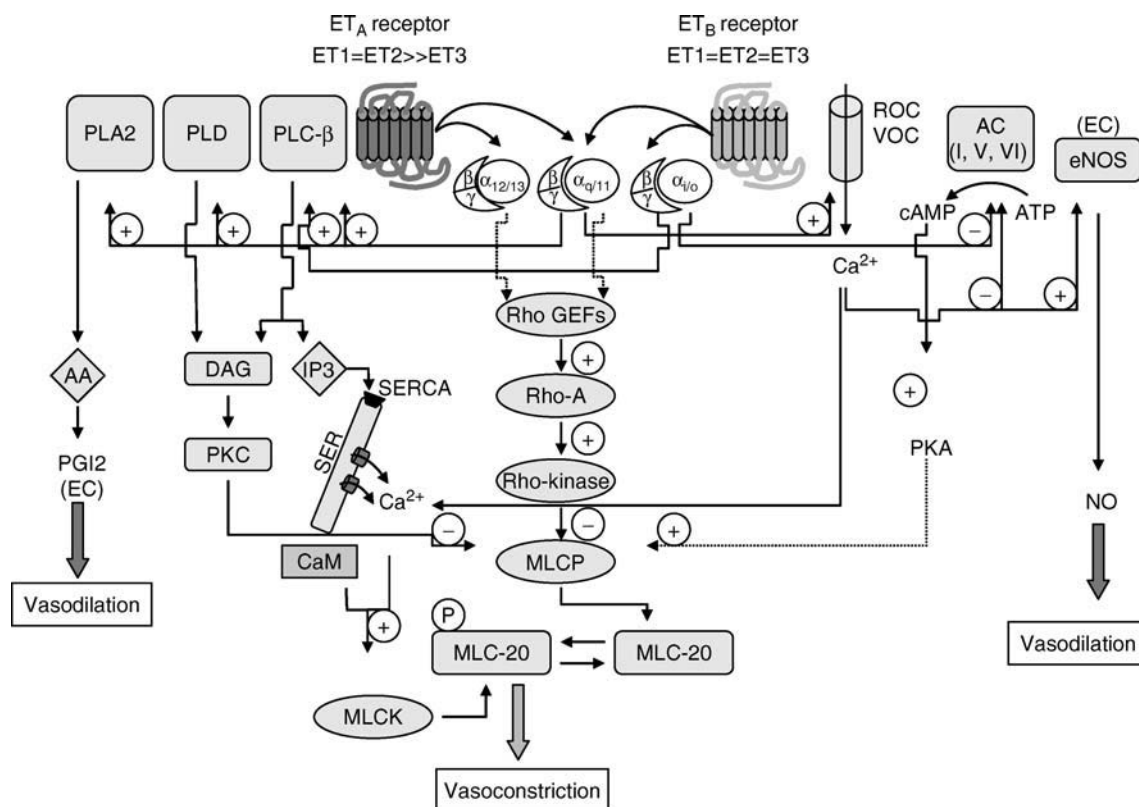
The endothelin receptor subtypes show differences in their signal transduction, ligand binding and tissue distribution. The ET_A receptor is isopeptide-selective and binds ET-1 and ET-2 with the same and ET-3 with 70–100-fold lower affinity. The ET_B receptor binds all three isoforms with the same affinity.

Pharmacological studies provided evidence for two subtypes of ET_A (ET_{A1}, ET_{A2}) and ET_B receptors (ET_{B1}, ET_{B2}), although genetic studies revealed only two different genes. Thus, the additional receptor subtypes may be derived from (i) alternative splicing, (ii) differences in post-translational processing or (iii) protein–protein interactions. Splice variants have been described for the ET_B receptor. However, these isoforms most likely do not account for the postulated second receptor subtype. One splice variant harbours ten additional amino acids in the third intracellular loop and has normal binding characteristics and functional activity (IP and cAMP formation). The second splice variant, which carries a completely altered intracellular C terminus, has normal binding properties but lacks functional activity. In vitro, homo- and heterodimerization of endothelin receptor subtypes has been demonstrated, but these receptor–receptor interactions are unlikely to account for the observed subtypes of ET_A

and ET_B receptors: Pharmacological binding profiles of ET_A/ET_B receptor heterodimers were essentially similar to those of individually expressed ET_A and ET_B receptors.

The ET_A receptor activates G proteins of the G_{q/11} and G_{12/13} family. The ET_B receptor stimulates G proteins of the G_i and G_{q/11} family. In endothelial cells, activation of the ET_B receptor stimulates the release of NO and prostacyclin (PGI₂) *via* pertussis toxin-sensitive G proteins. In smooth muscle cells, the activation of ET_A receptors leads to an increase of intracellular calcium *via* pertussis toxin-insensitive G proteins of the G_{q/11} family and to an activation of Rho proteins most likely *via* G proteins of the G_{12/13} family. Increase of intracellular calcium results in a calmodulin-dependent activation of the myosin light chain kinase (MLCK, Fig. 2). MLCK phosphorylates the 20 kDa myosin light chain (MLC-20), which then stimulates actin–myosin interaction of vascular smooth muscle cells resulting in vasoconstriction. Since activated Rho

E



Endothelins. Figure 2 Summary of short-term signalling events mediated by endothelin. In endothelial cells (EC) the activation of the ET_B receptor leads to the formation of prostacyclin (PGI₂) and NO, thereby eliciting vasodilation. In vascular smooth muscle cells activation of the ET_A receptor results in a robust vasoconstriction by a dual regulation of the 20 kDa myosin light chain (MLC-20). AA: arachidonic acid, AC: adenylyl cyclase, ATP: adenosine triphosphate, CaM: Calmodulin, cAMP: cyclic adenosine monophosphate, DAG, diacyl glycerol, eNOS: endothelial NO synthase, IP3: inositol-3,4,5-phosphate, MLC-20: 20 kDa myosin light chain, MLCP: myosin light chain phosphatase, Rho-GEF: Rho-guanine nucleotide exchange factor, SER: smooth endoplasmic reticulum, IP3R: IP3 receptor, PKA: protein kinase A, PKC: protein kinase C, PLA: phospholipase A, PLC: phospholipase C, PLD: phospholipase D.

inhibits the myosin light chain phosphatase *via* Rho-kinase, the dephosphorylation of the MLC-20 is blocked. The dual action of the ET_A receptor signalling on MLC-20 results in a robust vasoconstriction of vessels. Beside the short-term effects, such as vasodilation and vasoconstriction, endothelin receptors also stimulate long-term events (cell growth and differentiation). Whilst stimulation of the ET_A receptor results in a transient, monophasic activation of ERK1/2 *via* a G_{q/11}-proteins, activation of the ET_B receptor elicits a long-lasting, bisphasic ERK1/2 activation involving a G_{q/11}- and a G_i-mediated pathway (Fig. 3).

ET-1 also stimulates anti-apoptotic signal cascades in fibroblasts, vascular smooth muscles and endothelial cells (*via* phosphatidylinositol-3-kinase and Akt/protein kinase B). In prostate and ovarian cancer, upregulation of endothelin synthesis and ET_A receptors has been associated with a progression of the disease. The inhibition of ET_A receptors results in a reduced tumour growth. In malignant melanoma, ET_B receptors are associated with tumour progression. Endothelins can also stimulate apoptosis in stretch-activated vessels *via* the ET_B receptor, which contrasts the above-mentioned effects. The molecular basis for these differential anti- and pro-apoptotic reactions mediated by endothelins remains elusive.

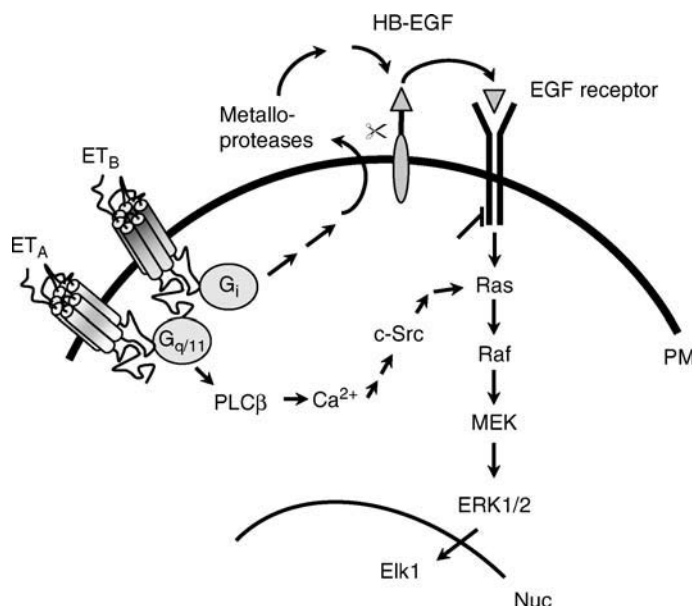
Activation of matrix metalloproteinases (MMP) is also involved in vascular and cardiac remodelling. For example, the fibrillar collagen matrix of the heart

maintains the shape of the left ventricle. If the delicate balance between matrix deposition and degradation is altered, cardiac fibrosis (increase of collagen synthesis) or left ventricular remodelling (increase of degradation) occurs. Activation of ET_A receptors leads to a stimulation of MMP-1, -2 and -9 in isolated myocytes and in the myocardium and thereby contributes to ventricular remodelling after myocardial infarction.

Sites of endothelin-receptor expression. ET_A receptors are expressed in the smooth muscle cells of the vascular medial layer and the airways, in cardiac myocytes, lung parenchyma, bronchiolar epithelial cells and prostate epithelial cells. ET_B receptors are expressed in endothelial cells, in bronchiolar smooth muscle cells, vascular smooth muscle cells of certain vessels (e.g. saphenous vein, internal mammary artery), in the renal proximal and distal tubule, the renal collecting duct and in the cells of the atrioventricular conducting system.

In addition, ET_B receptors are upregulated in vessels with atherosclerotic lesions and in pulmonary vessels of patients with severe pulmonary hypertension. The upregulation can be attributed to increased ET_B receptor expression in smooth muscle cells and to ET_B receptors expressed on infiltrating macrophages.

In the vascular system, endothelial ET_B receptors mediate a transient vasodilation, whereas ET_A receptors cause a long-lasting vasoconstriction. The role of ET_B receptors expressed on smooth muscle cells



Endothelins. Figure 3 Diagram of ET_A and ET_B receptor-mediated ERK1/2 activation. ET_A and ET_B receptor stimulate a transient ERK1/2 activation *via* G proteins of the G_{q/11}/PLCβ/Ca²⁺/c-Src-dependent pathway. In addition, the ET_B receptor stimulates a second, long-lasting phase of ERK1/2 activation *via* a G_i protein/metalloprotease/HB-EGF/EGF receptor-dependent pathway. *ERK2*: extracellular signal-regulated kinase; *HB-EGF*: heparin-binding epidermal growth factor; *Nuc*: nucleus; *PM*: plasma membrane.

remains elusive. In some vessels, ET_B receptor stimulation causes vasoconstriction which is, however, only of transient nature and quantitatively much lower than that following ET_A receptor activation. In the kidney, ET_A receptors, which are almost exclusively expressed in vessels, regulate renal circulation, whereas ET_B receptors expressed in the proximal and distal tubule and in the collecting duct are involved in natriuresis and diuresis. The main natriuretic action occurs most likely *via* the inhibition of the ▶**amiloride-sensitive Na⁺ channel** (ENaC). In the lung, endothelin causes long-lasting vaso- and bronchoconstriction. The contribution of each receptor subtype to the endothelin-evoked pulmonary responses is still controversial. The current data suggest that in the healthy lung ET_A receptors are involved primarily in pulmonary vasoconstriction and ET_B receptors in bronchoconstriction. In the central nervous system, ET_A receptors are expressed on smooth muscle cells of large- and small-cerebral arteries. In addition, ET_A receptors were also found to be expressed on endothelial cells isolated from capillaries and larger microvessels of the brain, but the physiological role of ET_A receptor expression in endothelial cells in the brain remains elusive. Further, ET_A receptors are expressed in small sensory C fibres. Activation of ET_A receptors in C fibres causes a sensitization of the tetrodotoxin-insensitive sodium channel as well as of the polymodal receptor TRPV1. Interestingly, stimulation of ET_B receptors expressed in keratinocytes leads to the release of β -endorphins, thereby counteracting ET_A receptor-mediated [3]. Neurons, particularly those of the level III and IV of the cortex predominately express ET_B receptors. In isolated astrocytes, both receptor subtypes were found to be expressed at the cell surface.

Genetic Studies and Human Diseases

Mice homozygous for an ET_A receptor gene disruption show craniofacial malformations, such as cleft palate, micrognathia, microtia and microglossia. ET_A (–/–) mice die shortly after birth due to respiratory failure. Mice with an ET-1-null mutation show the same craniofacial malformations and, in addition, cardiovascular disorders (e.g. septal defects, abnormal cardiac outflow tract, aortic arch and subclavian arteries).

Mice with a disruption of the ET-3 or the ET_B receptor gene display pigment disorder and a megacolon. The former resembles the congenital megacolon associated with pigment disorders and cochlear hearing problems (Waardenberg syndrome) or the isolated congenital megacolon (Hirschsprung's disease) observed in man. Hirschsprung's disease and Waardenberg syndrome can be caused by several different gene mutations, among others inactivating mutations in the ET-3 and the ET_B receptor gene (about 5% of the patients with

Hirschsprung's disease have ET_B receptor mutations). The lack of ET-3/ET_B receptor results in the absence of parasympathic ganglionic neurons in the myenteric plexus (Auerbach). Mice with an ET-3/ET_B receptor disruption die within 2 weeks after birth. In transgenic mice, in which the expression of the ET_B receptor is driven by the dopamine β -hydroxylase promoter, normal myenteric plexus are present and no enteric disorder develops. These mice, however, show a salt-sensitive hypertension, which can be efficiently treated with amiloride, indicating that ET_B receptors are involved in the regulation of natriuresis *via* the amiloride-sensitive sodium channel ENaC.

The genetically engineered disruption of the ECE1 gene causes craniofacial and cardiovascular malformations (ET-1/ET_A receptor phenotype), congenital megacolon and pigment disorders (ET-3/ET_B receptor phenotype). The ECE-2 (–/–) mice do not display any abnormality, indicating that ECE-1 is of crucial importance in embryonic development. Strikingly, ECE-1 (–/–) mice and ECE-1 (–/–)/ECE-2 (–/–) mice still have about 60% of wild-type ET-1 levels [4]. This result indicates that alternative pathways in the generation of mature ET-1 exist (e.g. NEP). This alternatively generated ET-1, however, cannot compensate for the embryogenic defects.

ECE isoforms are also involved in the degradation of A β peptide. A genetic variant of ECE-1 with an increased transcriptional activity is associated with a decreased risk for AD. Thus, the inhibition of ECE in the CNS may increase the risk for the development of Alzheimer's disease (AD).

In malignant prostate epithelial cells, auto- and paracrine release of ET-1 is a critical factor in ET_A receptor-mediated proliferation [5]. In addition, the ET-1/ET_A receptor axis has emerged as a potential target in prostate cancer bone metastasis

Drugs

Clinical Use

As endothelins mediate potent vasoconstrictor effects, ECE inhibitors and endothelin receptor antagonists were developed for the treatment of cardiovascular diseases, such as acute and chronic heart failure, pulmonary hypertension and subarachnoid haemorrhage. As ET_A receptors have potent mitogenic responses and may promote progression of ovarian and prostate cancer and bone metastases ET_A receptors are also considered as a potential targets for anti-tumour activity.

A great number of ECE-inhibitors and mixed and selective ET_A and ET_B receptor antagonists have been developed in the past. For specific inhibitors of ECE, however, only very limited effects on the endothelin system were found. The limited potency of ECE inhibition might be due to the generation of mature

Endothelins. Table 3 Summary of clinical trials with endothelin receptor antagonists or ECE-inhibitors

Drug	Company	Antagonist/ Inhibitor	Approval/clinical study
Bosentan (Tracleer®)	Actelion, Switzerland	ET _A /ET _B re- ceptor	Grade III and IV PAH and chronic thromboembolic pulmonary hypertension (European approval in 2002 as orphan medicine) Program for CHF was terminated in phase III
TBC11251 (Sitaxsentan)	Texas Biotech- nology, USA	ET _A receptor	Grade III PAH (designated orphan medicine in 2004)
Ambrisentan (Letairis)	Gilead, USA	ET _A receptor	Grade II and III PAH (designated orphan medicine in 2005, US approval in 2007)
ABT-627 (Atrasentan)	Abbott Labora- tories, USA	ET _A receptor	Hormone-refractory prostate cancer, stage IV (phase III)
S-0139	Shionogi/GSK, Japan	ET _A receptor	Hemorrhagic and ischemic stroke (phase II, Japan)
SLV306	Solvay, Germany	NEP/ECE (dual inhibitor)	Hypertension and diabetic nephropathy (phase II)
Ro-61–0612 (Tezosentan)	Actelion, Switzerland	ET _A /ET _B re- ceptor	AHF (phase III, discontinued)
LU135252 (Darusentan)	Aventis, Germany	ET _A receptor	CHF (phase III, discontinued)
ZD4054	AstraZeneca, UK	ET _A receptor	Hormone-refractory prostate cancer (phase II)

AHF: acute heart failure, CHF: chronic heart failure, CRF: chronic renal failure, NEP: neutral endopeptidase, ECE: endothelin converting enzyme, PAH: pulmonary arterial hypertension.

ET-1 from big-ET-1 by other proteases such as neutral endopeptidase or other currently unidentified proteases. Therefore, dual inhibition of ECE and NEP might inhibit ET-1 generation more efficiently, than that seen for selective ECE inhibitors. However, dual inhibition of ECE and NEP could also increase the risk for the development of AD, as both enzyme classes are involved in the degradation of Aβ peptide.

In the case of receptor antagonists, it is still unknown whether mixed antagonism of endothelin receptors or selective blockade of ET_A receptors is of greater benefit in the treatment of diseases. Several clinical trials have been launched involving the treatment of heart and renal failure, pulmonary hypertension, subarachnoid haemorrhage and prostate cancer (Table 3). The majority of the studies is performed with selective ET_A receptor antagonists. Recently, the petidic ETB receptor agonist SPI-1620 (Spectrum Pharmaceuticals) has entered clinical development as an adjunct for chemotherapy (increasing tumour blood flow and delivery of anti-cancer drugs). At present, however, only the mixed endothelin receptor antagonist Tracleer (Bosentan) and the ETA receptor-selective antagonist Letairis (Ambrisentan) are approved for treatment (pulmonary hypertension).

- Blood Pressure Control
- Metalloproteinase

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Endothelium

The cell layer lining blood vessels. It is now well established that endothelial cells not only maintain a non-thrombogenic surface in the blood vessels, but also

perform a variety of other functions such as regulation of blood pressure and production of tissue-specific growth factors.

- ▶ Endothelins
- ▶ Vascular Endothelial Growth Factor
- ▶ Nitric Oxide

Endothelium-derived Relaxing Factor (EDRF)

Vasodilating molecule(s) liberated from vascular endothelial cells in response to chemical substances (i.e., Acetylcholine, bradykinin, substance P, etc.) or mechanical stimuli (i.e., shear stress, transmural pressure, etc.). The EDRF includes NO, prostaglandin I₂ (prosta-cyclin), and endothelium-derived hyperpolarizing factor (EDHF).

- ▶ Nitric Oxide

Endotoxin

Endotoxins are the lipopolysaccharides (LPS) of the outer membrane of Gram-negative bacteria. They trigger inflammatory reactions in the infected organism, activate complement and cause fever or even a septic shock. They act on toll-like receptors.

- ▶ Toll-like Receptors

Energy Balance

Homeostatic regulation of metabolic efficiency (i.e., caloric intake required to maintain bodyweight constant to maintain constant ratios of energy expenditure/conservation).

- ▶ Appetite Control
- ▶ Orexins
- ▶ Fatty Acid Transport Proteins

Enkephalin

Enkephalins belong to the group of endogenous opioid peptides.

- ▶ Opioid System
- ▶ Analgesics

Entamoeba Histolytica

Entamoeba histolytica is an anaerobic rhizopod that occurs in tropical and subtropical areas. It can cause intestinal and extraintestinal manifestations. It is transmitted orally by ingestion of cysts that develop into trophozoites in the large intestine. Amebic trophozoites release several cytolytic factors, e.g. amoebapore, which enable the parasite to invade tissue. In intestinal amoebiasis, *E. histolytica* trophozoites invade the intestinal mucosa, causing a form of ulcerative colitis with bloody and mucous diarrhoea. Extraintestinal manifestation of amoebiasis results in abscess formation, usually in the liver but sometimes in the brain.

- ▶ Antiprotozoal Drugs

Envelope (Viral)

In some viruses, the capsid is surrounded by a lipid membrane (envelope), which is derived from the host cell membrane at the site of virus budding. The membrane contains viral envelope glycoproteins as well as host cell membrane proteins.

- ▶ Antiviral Drugs
- ▶ Interferons
- ▶ Viral Proteases

Eosinophil

- ▶ Allergy
- ▶ Immune Defense
- ▶ Leukotrienes

Epacs

Epacs are guanine nucleotide exchange factors (GEFs) for small G-proteins (e.g. Rap1, Rap2, Rim1, Rim2) and are directly activated by cyclic AMP. cyclic AMP binds Epacs through a motif homologous with that of the regulatory subunits of PKA and cyclic nucleotide gated channels. Half-maximal activation occurs at $\sim 40\mu\text{M}$, as compared with activation of protein kinase A at $\sim 1\mu\text{M}$.

- ▶ Cyclic Adenosine Monophosphate
- ▶ Adenylyl Cyclases

Eph Receptor Tyrosine Kinase

- ▶ Angiogenesis and Vascular Morphogenesis
- ▶ Table appendix: Receptor Proteins

Ephrins

Ephrins are a group of membranous ligands, which function through a family of receptor tyrosine kinases (Ephs). Ephrin/Eph-mediated signaling processes are involved in morphogenetic processes taking place e.g. during the development of the nervous system or the vasculature.

- ▶ Angiogenesis and Vascular Morphogenesis

Epidermal Growth Factor (EGF)

- ▶ Growth Factors

Epidermal Growth Factor (EGF) Receptor Family

The epidermal growth factor (EGF) receptor family, also called the “ErbB-receptor family,” consists of four receptor family members ErbB1 (EGFR), ErbB2 (Neu, HER2), ErbB3 (HER3), and ErbB4 (HER4).

ErbB-receptors can form heterodimers and mediate the actions of a variety of factors including epidermal growth factor (EGF), epiregulin, or neuregulins. ErbB2-containing receptors are highly upregulated in a variety of tumors.

- ▶ Growth Factors
- ▶ Receptor Tyrosine Kinases
- ▶ Targeted Cancer Therapy
- ▶ Table Appendix: Receptor Proteins

Epidermal Growth Factor Receptor 2 (ErbB2)

The epidermal growth factor receptor 2 (HER-2) is a protein found on the surface of cells. Heterodimerization of HER-2 activates the enzyme tyrosine kinase, triggering reactions that cause the cells to grow and multiply. HER-2 is found at abnormally high levels on the surface of many types of cancer cells, which may divide excessively. Antibodies targeting HER-2 (e.g., trastuzumab) are used as antineoplastic agents.

- ▶ Targeted Cancer Therapy
- ▶ Growth Factors

Epidural (Space)

The epidural space surrounds the dura mater of the spinal cord. It is bounded by the pedicles of the vertebral arches and by the anterior and posterior ligaments connecting the bony vertebral column. The epidural space contains nerve roots, fat, and blood vessels.

- ▶ Local Anaesthetics

Epilepsy

Epilepsy is a heterogeneous group of syndromes characterized by abnormal, rhythmic electrical activity of the brain or parts of the brain. The term ‘epilepsy’ is reserved for chronic diseases, while a single, isolated seizure does not justify the diagnosis of epilepsy.

- ▶ Antiepileptic Drugs

Epinephrine

► Adrenaline

Episodic Ataxia/Myokymia

Episodic ataxia (EA) is an autosomal dominant disorder that brief episodes of ataxia can be triggered by physical or emotional stress. The symptom can occur several times during the day, last for seconds to minutes, and be associated with dysarthria and motor neuron activity, which causes muscle rippling (myokymia) between and during attacks. It is caused by a mutation in a neuronal voltage dependent Ca²⁺ channel.

► Voltage-dependent Ca²⁺ Channels

Episome

An episome is nuclear DNA that is maintained without integrating into chromosomes.

► Gene Therapy

Epithelial Ca²⁺ Channel

Epithelial calcium channel 1 (ECaC1), synonym TRPV5, is a member of the TRP family of ion channels, implicated in vitamin D-dependent transcellular Ca²⁺ transport in epithelial cells of the kidney, placenta and the intestine.

► TRP Channels

Epithelial Mesenchymal Transition (EMT)

A process by which epithelial cells lose cell–cell adhesion and become more migratory and acquire mesenchymal properties. The process is associated with

drastic changes in gene expression, leading to the downregulation of epithelial marker genes such as E-cadherin, claudins, and keratin, and the upregulation of mesenchymal markers such as vimentin. EMT occurs during embryonic development, e.g. during gastrulation, and neural crest formation, and carcinoma invasion. The reverse of EMT, mesenchymal-to-epithelia transition is observed for instance during formation of kidney tubules from mesenchymal precursor cells, but frequently also when carcinoma cells form a metastasis in the target organ.

► Cadherins/Catenins

Epithelial Na⁺ Channel

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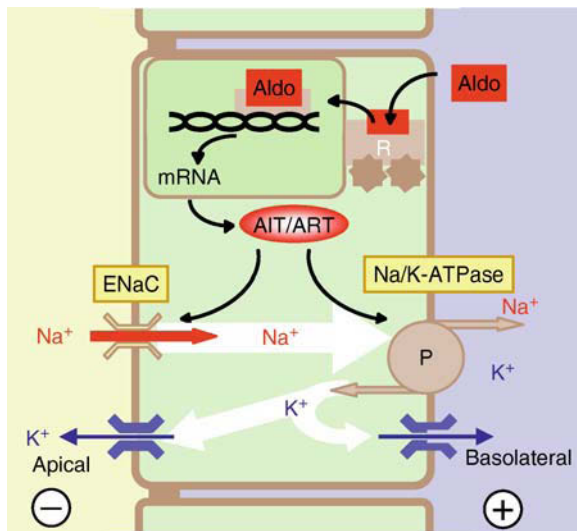
Definition

The epithelial Na⁺ channel (ENaC) is the highly selective Na⁺ channel expressed in epithelia. The ENaC channel is a heteromeric channel made of homologous α β γ ENaC subunits. The human genes encoding the α β γ ENaC named *SCNN1A*, *SCNN1B*, and *SCNN1G* are located in chromosome 12 (α) and 16 (β γ), respectively. The mouse genes encoding the α β γ ENaC named *Scnn1a*, *Scnn1b*, and *Scnn1g* are located on chromosomes 6 (α) and 7 (β γ), respectively [1].

Basic Characteristics

ENaC belongs to a recently discovered family of ► **ionic channels** that include in mammals the neuronal acid-sensing ion channels (ASICs) and in the worm *C.elegans* the degenerin channel family (Mec4, Mec10) involved in mechanosensation [2]. The homologous α β γ subunits of ENaC consist of a large extracellular loop, two transmembrane domains, and short intracellular N- and C-termini, and are arranged pseudosymmetrically around the channel pore.

ENaC is located in the apical membrane of polarized epithelial cells where it mediates Na⁺ transport across tight epithelia [3]. The most important tight epithelia expressing ENaC include the distal nephron of the kidney, the respiratory epithelium, and the distal colon. The basic function of ENaC in polarized epithelial cells is to allow vectorial transcellular transport of Na⁺ ions. This transepithelial Na⁺ transport through a cell involves



Epithelial Na⁺ Channel. Figure 1 Transepithelial ion transport in a principal cell of the aldosterone-sensitive distal nephron (ASDN).

basically two steps, as illustrated in the Fig. 1. The large electrochemical gradient for Na⁺ ions existing across the apical membrane provides the driving force for the entry of Na⁺ into the cell. Active Na⁺ transport across the basolateral membrane is accomplished by the ►Na⁺/K⁺-ATPase.

ENaC mediates Na⁺ entry from the tubule lumen at the apical membrane and the Na⁺/K⁺ ATPase extrudes Na⁺ at the basolateral side. K⁺ channels are present on the basolateral and apical membrane. K⁺ channels at the apical membrane mediate K⁺ secretion into the tubular lumen.

In the *aldosterone-sensitive distal nephron* (ASDN), ENaC-mediated Na⁺ absorption under the control of aldosterone is critical to balance urinary Na⁺ excretion with the daily intake. ENaC allows Na⁺ entry from the tubule lumen at the apical membrane, and the Na⁺/K⁺ ATPase extrudes Na⁺ at the basolateral side. The Na⁺ absorption in the distal nephron is coupled to K⁺ secretion via K⁺ channels (ROMK2) located at the apical membrane.

Three successive tubule portions contribute to the ASDN: the late portion of the distal convoluted tubule, the connecting tubule, and the collecting duct. The recent observation that collecting duct-specific inactivation of α ENaC in the mouse kidney does not impair sodium and potassium balance, suggests that the more proximal nephron segments (late distal convoluted tubule, connecting tubule) are mainly important for achieving sodium and potassium balance.

ENaC activity is under the control of ►aldosterone and ►vasopressin that are secreted in response to stimuli

such as extracellular volume contraction, dehydration, or hyperkalemia. Aldosterone binds to intracellular mineralocorticoid receptors (MR receptors); the ligand–receptor complex is translocated to the nucleus and induces the expression of ENaC and the Na⁺/K⁺-ATPase proteins via aldosterone-induced transcripts (AITs) and/or aldosterone-repressed transcripts (ARTs). Vasopressin binds to the G-protein coupled V2 receptor and activates ENaC via the c-AMP-dependent pathway.

The role of ENaC is crucial for the maintenance of the extracellular fluid volume and blood pressure [4]. The recent identification of mutations in genes encoding the ENaC, the mineralocorticoid receptor (MR) and the ►11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD-2) that cause monogenic forms of hypertension, strongly supports this notion. Mutations of ENaC associated with an increased Na⁺ absorption in the distal nephron leading to a low aldosterone and low plasma renin hypertension (►Liddle syndrome or pseudoaldosteronism), are activating mutations that remove a conserved proline-rich motif in the intracytoplasmic region of ENaC, important for channel endocytosis and degradation. Conversely, loss of function mutations cause Na⁺ salt losing nephropathy with dehydration, hyperkalemia, elevated plasma renin, and aldosterone levels characteristic of the recessive form of pseudohypoaldosteronism Type 1 (recessive PHA-1).

In the lung, ENaC is important for the ionic composition and the clearance of the ►airway surface liquid (ASL) [5]. The activity of ENaC is inversely coupled to that of CFTR (Cystic fibrosis transmembrane regulator), responsible for chloride secretion at the apical membrane or airway epithelium. In cystic fibrosis, CFTR activity is lost and ENaC activity enhanced, causing an increased fluid reabsorption of ASL and increased mucous viscosity, preventing a normal mucociliary clearance. In mice, airway-specific overexpression of ENaC results in an increased Na⁺ absorption and in a severe spontaneous lung disease sharing features with cystic fibrosis. Conversely, loss of function mutations of ENaC, as observed in recessive PHA-1, cause an increase of mucociliary clearance and a decrease in mucous viscosity. ENaC activity in the lung is developmentally regulated and plays a critical role at birth for a rapid and normal fluid reabsorption from the lung which turns from a secretory (fetal) to a reabsorptive (adult) mode. Respiratory distress syndrome (RDS) observed in premature newborn can be, in part, due to ENaC immaturity. In the fetal lung, ENaC maturation is controlled by glucocorticoids and, at birth, by sympathicomimetics and pO₂. In the adult distal lung, ENaC activity is primarily controlled by ► β adrenergic agonist and pO₂. It plays an important role in the pathophysiology of high altitude pulmonary

edema (HAPE), which can be greatly prevented by administration of β adrenergic drugs.

In the *skin*, ENaC is expressed in keratinocytes of the epidermis and in hair follicles. It could play a role in terminal differentiation by modulating keratinocyte calcium signaling. The skin expresses MR, GR, and 11β HSD2, but the role of aldosterone and glucocorticoids on ENaC activity and keratinocyte differentiation is not yet understood.

In the *tongue*, ENaC is expressed in *taste bud* epithelial cells. The expression of α , β and γ subunits at the apical membrane of taste buds is observed under low salt diet, known to greatly increase plasma aldosterone. This observation suggests that ENaC could play a significant role in the transduction of salt sensation.

In the *inner ear*, ENaC is expressed in supporting cells surrounding **►hair cells** and is postulated to play a role in the low sodium concentration of endolymph, critical for proper mechanotransduction and hearing.

Drugs

ENaC is blocked from the tubular lumen of the distal nephron by amiloride at submicromolar concentrations. By blocking ENaC activity at the apical membrane of the target cell of the distal nephron, the electrochemical gradient favoring the passive secretion of potassium through ROMK2 (or other apically located K channels) is dissipated and potassium secretion blocked. Unlike loop diuretics (**►furosemide** and analogs) or distal convoluted diuretics (i.e., **►thiazide** diuretics), which cause potassium secretion and **►hypokalemia**, amiloride and analogs cause potassium retention (and ultimately **►hyperkalemia**) and are currently used as K^+ -*sparing* diuretics. Spironolactone, a competitive antagonist of aldosterone for the mineralocorticoid receptor, inhibits epithelial sodium transport and potassium secretion and belongs to the same class of diuretic. Obviously, it is mostly effective when plasma aldosterone is elevated (**►primary or secondary aldosteronism**), whereas amiloride is effective even in the presence of low circulating aldosterone. The biophysical characteristics of the block of ENaC by amiloride including voltage-dependence, competitive interaction with permeant cations, such as Na^+ or Li^+ ions, strongly suggest that amiloride is a pore blocker which, upon binding to its receptor on the channel, physically occludes the ion permeation pathway. The binding site for amiloride is located in the extracellular vestibule of the channel pore close to the ion selectivity filter and involves specific amino acid residues on each the extracellular domain of the α β γ ENaC subunits. Amiloride shares its binding site on ENaC with triamterene which blocks the channel with a lower affinity.

Since the distal nephron reabsorbs under physiological conditions less than 10% of the filtered load of

sodium, blockade of ENaC results only in a slight increase in urinary excretion of sodium. Consequently, amiloride is usually used in association with other diuretics. As suggested by genetic forms of hypertension associated with ENaC gain of function mutations, amiloride should be efficient in the treatment of low-renin, salt-sensitive hypertension but, unfortunately, its potency is markedly lowered by salt intake. The development of “*noncompetitive*” antagonists of ENaC (i.e., potent in the presence of high luminal sodium) would be a useful addition to the drugs available today.

The development of amiloride analogs targeted to block selectively ENaC in the lung could be useful in the treatment of **►cystic fibrosis (CF)** patients or more generally of patients suffering from chronic bronchitis, a condition in which an increased mucociliary clearance is highly desirable. *Channel activators* are presently not available and will be useful for the treatment of RDS in newborn or HAPE in adult, or, possibly, lung edema in congestive heart failure.

►Gluco-mineralocorticoid Receptors

►Diuretics

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Epitope

Regions of a molecule that can be recognized by an antibody or T cell receptor.

►Immune Defense

►T Cell Receptors

EPS

Extrapyramidal Side Effects.

- ▶ Antipsychotic Drugs
- ▶ Dopamine System

EPSP

Synonyms

Excitatory Postsynaptic Potential

Definition

A postsynaptic potential or PSP is the voltage response of a postsynaptic neuron to a neurotransmitter released by a nerve terminal. The response may be depolarizing, in which case the voltage shift is in a positive direction causing an excitatory effect or EPSP, or hyperpolarizing, in which case the voltage shift is in a negative direction causing an inhibitory effect or IPSP.

- ▶ Synaptic Transmission
- ▶ Ionotropic Glutamate Receptors
- ▶ Psychotomimetic Drugs

ER

Endoplasmic reticulum.

- ▶ Protein Trafficking and Quality Control
- ▶ Intracellular Transport
- ▶ Chaperones

ER/Golgi Intermediate Compartment (ERGIC)

Highly mobile tubovesicular structures located in the vicinity of the Golgi apparatus and also in the periphery of the cell (also known as VTCs = vesicular tubular

clusters). The ERGIC receives proteins from the ER and delivers them to the Golgi apparatus. The ERGIC concentrates proteins in the secretory pathway and is also part of the quality control system.

- ▶ Protein Trafficking and Quality Control

ERAD

ER-Associated Degradation, when proteins mis-fold in the ER due to mutation or environmental conditions, they are selectively exported to the cytosol for degradation by the proteasome.

- ▶ Chaperones

ErbB Receptor Family

- ▶ Epidermal Growth Factor Receptor Family
- ▶ Growth Factors
- ▶ Table appendix: Receptor Proteins

Erectile Dysfunction

Erectile dysfunction is treated by local or systemic application of vasodilator drugs. The most effective is sildenafil, a phosphodiesterase-type 5 inhibitor.

- ▶ Phosphodiesterases
- ▶ Smooth Muscle Tone Regulation

ERGIC

- ▶ ER/Golgi Intermediate Compartment
- ▶ Protein Trafficking and Quality Control

Ergot Alkaloids

Ergot alkaloids occur naturally in the fungus *Claviceps purpurea* which infects cereal crops. Contaminated grain has caused epidemics of ergot poisoning for centuries. The key symptom of ergot poisoning is irreversible, painful peripheral vasoconstriction leading to peripheral gangrene (St. Anthony's fire). Ergot alkaloids are a rather heterogeneous group acting on adrenoceptors, dopamine receptors and 5-HT (serotonin)-receptors. Examples are ergotamine, dihydroergotamine, bromocryptine and methysergide. They are used in the prophylaxis and treatment of migraine, the treatment of parkinsonism and in the prevention of postpartum haemorrhage.

- ▶ α -Adrenergic System
- ▶ Dopamine System
- ▶ Serotonergic System

ERT

- ▶ Estrogen Replacement Therapy

Erythropoietin

Erythropoietin is a growth factor produced by interstitial cells of the kidney in response to hypoxia. Erythropoietin stimulates haematopoiesis in the bone marrow. Recombinant human erythropoietin is used to treat anemias, e.g. anemia caused by chronic renal failure and anemia in AIDS and cancer patients.

- ▶ Hematopoietic Growth Factors
- ▶ Cytokines

ESI-MS

Electrospray Ionization Mass Spectrometry.

- ▶ Proteomics

EST

Expressed Sequence Tag. A short DNA sequence usually representing the most terminal regions of a cDNA clone.

- ▶ Microarray Technology

Estrogen Receptor

- ▶ Selective Sex Steroid Receptor Modulators
- ▶ Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptors

Estrogen Replacement Therapy (ERT)

ERT is a clinical treatment whereby premenopausal estrogen levels are restored and the symptoms of menopause are relieved.

- ▶ Selective Sex Steroid Receptor Modulators
- ▶ Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptors

Estrogens

Estrogens are 18-carbon steroids based on the estrane nucleus. In the non-pregnant female, they are mainly produced in the ovary.

- ▶ Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptors
- ▶ Contraceptives
- ▶ Selective Sex-Steroid Receptor Modulators

Ethanol

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Synonyms

Ethyl alcohol

Definition

C_2H_5OH , ►ethanol is formed by bacteria in the gastrointestinal tract in low amounts. Most of the ethanol of bacterial source is metabolized during the first liver passage yielding acetaldehyde and subsequently acetic acid.

Mechanism of Action

The main target structures in the brain that are affected by ethanol are ►GABA_A-receptors and glutamate-►NMDA-receptors. The action of the neurotransmitter γ -amino-butyric acid (►GABA) is facilitated, and the function of the NMDA-receptor is reduced by physiologically relevant concentrations of ethanol (10–50 mM). Therefore, this essay focuses on GABA and glutamate. Somewhat higher doses of ethanol affect other neuronal systems, among them endogenous opioids, dopamine and serotonin. Furthermore, second messenger and other intracellular mechanisms are altered after both acute and chronic exposure. Chronic ethanol abuse causes severe health problems. This essay does not deal with these aspects.

►GABA_A receptor: The inhibitory GABA_A and strychnine-sensitive glycine receptors are modulated positively by ethanol. Site-directed mutagenesis techniques have identified amino acid residues important for this action. Mutation of a single amino acid in specific transmembrane domains (TM2 (Ser270) and TM3) of the α_2 and β subunits of GABA_A and glycine (TM2) receptors abolish the action of ethanol. Initial studies of transgenic mice with a reduced sensitivity to ethanol as measured by loss of righting reflex revealed an ethanol-resistant α_1 -subunit of the glycine receptor [2]. The corresponding mutations introduced into the γ subunit of GABA_A-R had less effect. These findings indicate that in the GABA_A receptor, ethanol may bind in a cavity formed between TM2 and TM3, and that binding to the α or β subunit may be more critical than to the γ subunit.

In an attempt to visualize the site of action of ethanol, tryptophan mutation at position S270, TM2 and TM3 domains of the GABA_A α_2 subunit were modeled as antiparallel α -helices. The model showed that the region between S270 TM2 and TM3 contains a small cavity that may not be filled by side chains of adjoining helices. In contrast, the model of the S270W mutation demonstrated that the side chain of tryptophan completely occupied this cavity, which could eliminate occupation of the putative cavity by ethanol.

These findings were unexpected because previous studies had demonstrated that the γ_2 subunit is required for potentiation of GABA_A receptor function by low concentrations of ethanol [2]. The γ_2 subunit gene is located within a definitely mapped quantitative trait locus (QTL) for acute alcohol withdrawal on mouse chromosome 11 [1]. Allelic variation was genetically

correlated with acute alcohol withdrawal, ethanol-conditioned taste aversion, ethanol-induced motor incoordination and ethanol-induced hypothermia [1].

Furthermore, the γ_2 subunit of GABA_A-R is the most abundant subunit in the central nervous system (CNS) and is required for localization to synapses. The γ_2 subunit exists as two spliced variants, the long version (γ_{2L}) containing an additional eight amino acids in the large TM3/4 intracellular loop, relative to the short version (γ_{2S}). The extrapeptide sequence contains a consensus sequence for ►protein kinase C (PKC) phosphorylation. The γ_{2L} subunit was at one time claimed to be essential for ethanol modulation of GABA_A-R function, presumably related to the unique PKC substrate on this subunit. Differential functions for γ_{2L} and γ_{2S} might involve rapid regulation of GABA_A-R channels by PKC. Another possibility is regulation of GABA_A-R subcellular targeting, trafficking, or turnover, presumably involving interactions with other proteins. Chronic ethanol induced a drop in the γ_{2L}/γ_{2S} ratio, e.g., lower levels of the γ_{2L} splice variant. This might favor production of $\alpha_4\beta_2\gamma_{2S}$ receptor composition. This subtype differs markedly in various properties (compared to $\alpha_4\beta\gamma_{2L}$) including sensitivity to zinc inhibition, channel kinetics, and possibly sensitivity to positive modulation by neurosteroids. PKC involvement in ethanol pharmacology and interactions with GABA_A-R is also supported by changes in ethanol sensitivity of mice lacking PKC γ and other subtypes of PKC. PKC γ null mutant mice displayed reduced sensitivity to the effects of ethanol on loss of righting reflex and hypothermia, and abolished the ethanol-enhancement of GABA_A receptor agonist muscimol stimulated ^{36}Cl -uptake, demonstrating at least the link between behavioral actions of ethanol, PKC phosphorylation, and GABA receptor function [2].

PKC ϵ knockout mice are supersensitive to acute low-dose hyperlocomotor and high-dose sedative effects of ethanol and other drugs such as diazepam and pentobarbital, which allosterically activate GABA_A receptors. In addition, these mice voluntarily consume 75% less alcohol than wild-type mice when tested by using a two-bottle choice paradigm. They also showed about 50% less alcohol-reinforced operant responses than wild-type mice and reduced relapse drinking after a period of alcohol deprivation. These findings were not associated with metabolic changes of ethanol nor with receptor binding affinity or density in cerebral cortex, striatum or cerebellum. These findings suggest that PKC ϵ regulates sensitivity to ethanol intoxication and thereby influences alcohol consumption. GABA_A receptors from PKC ϵ -null mice were more sensitive to activation by muscimol (GABA agonist) plus ethanol or flunitrazepam. PKC ϵ might regulate sensitivity of GABA receptors to allosteric activators possibly by phosphorylating the polypeptide.

Furthermore, PKC ϵ is required for nerve growth factor-induced activation of mitogen-activated protein kinases and neurite outgrowth by ethanol. It is also required for ethanol-induced increases in N-type voltage-gated calcium channels in PC12 neural cells.

In several studies, chronic ethanol treatment has been associated with PKC upregulation. In PC12 cells, increased levels of PKC δ and ϵ were found (25–200 mM ethanol, 2–8 days of treatment) which was associated with increased PKC-mediated phosphorylation.

The possibility that acute ethanol directly activates PKC would seem to be ruled out by the lack of such effect occurring in various *in vitro* systems that have been studied. One possibility is the activation of a phosphatase, others are the modulation of the availability and type of activator. It is also possible that ethanol could modify the sensitivity of the ion channel to the effect of PKC phosphorylation or its proteolytic downregulation.

Overall, our understanding of the precise location and substrates for the different PKC isoforms and protein–protein interactions involving PKC is still in its infancy.

Subunit changes are other mechanisms that alter the physiology of GABA synapses and account for plastic changes seen following chronic ethanol treatment.

Dependent on the various treatment regimes (continuous administration, chronic intermittent administration with multiple ethanol withdrawal, CIE) chronic ethanol produced reduced GABA $_A$ -R mediated synaptic inhibition, hyperexcitability and seizure susceptibility. The α_6 subunit polypeptide was increased in cerebellum after both regimes, although there was no significant increase in α_6 mRNA in CIE in contrast to continuous ethanol paradigms and a decrease in α_1 subunit mRNA. A 20–30% increase in α_4 subunit mRNA was detected in hippocampal formation in CIE treatment paradigm. Thus, the cerebellar changes occur with chronic ethanol no matter which paradigm is used and are transient. They might contribute to short-term plasticity such as tolerance to motor impairment. Reduced function and altered pharmacological properties of GABA-R in the hippocampus of CIE rats were more persistent, lasting at least 2 days in some cases up to 40 days. Thus, the changes in this region such as increased α_4 and γ_{2S} subunits might be more important in the altered behavior of CIE rats. Continuous ethanol also produced an increase in diazepam-insensitive binding (involving α_4 and α_6 subunits) in the cortex (specifically α_4 because α_6 is not found in cortex).

NMDA-Glutamate Receptor

The other important molecular target of ethanol is the $\blacktriangleright$ N-methyl-D-aspartate receptor (NMDA-R), which is acutely inhibited although the mechanism is not clear. It was speculated that at least in some brain region the coactivating glycine sites are involved and/or the coactivating polyamine sites. The receptors containing

the NMDA-R2B subunits are the most ethanol-sensitive [4]. The subunit has a fyn-kinase phosphorylation site that may rapidly render NMDA-R1 insensitive to ethanol during the development of acute tolerance.

NMDA-R play a major role in various aspects of chronic ethanol action, e.g., withdrawal and drug-dependent reorganization of neural circuitry [5]. Long-term potentiation (LTP) and long-term depression (LTD) are important candidate mechanisms for the drug-induced reorganization of neural circuitry that occurs during addiction. Both processes require activation of NMDA-R. One exception is the dorsal striatum in which the rise in Ca $^{2+}$ mediates the LTD by the activation of voltage dependent Ca $^{2+}$ channels. It is interesting to note that the numbers of both the NMDA-R and the voltage-dependent Ca $^{2+}$ channels (L-type, PKC δ -dependent, N-type, PKC ϵ -dependent) are increased after chronic ethanol [4]. This is paralleled by an increase in NMDA-R function as measured by an NMDA-induced increase in [21 Ca] influx. These events are present after 7 days of withdrawal and seem to be associated with lowered seizure threshold.

Distinct alterations in neural gene expression of NMDA-R1 splice variants and the NMDA-R2B subunit are observed after long-term ethanol ingestion. Increased mRNA levels of the NMDAR1–1 splice variant can be detected in all brain regions that expressed this isoform. On the other hand, the NMDA-R2B subunit decreases dramatically both at the mRNA and protein levels. However, 24 h after onset of withdrawal NMDA-R2B mRNA and hippocampal protein levels are elevated dramatically. Furthermore, NMDAR1–4 splice variant expression of mRNA and protein are elevated in the hippocampus 24 h after ethanol withdrawal. One may speculate that cells that express heterodimeric NMDA-R1/2B receptors are highly susceptible to ethanol. Another study found increases (–35%) in NR1, NR2A, and NR2B protein levels in homogenates from the cortex and hippocampus of rats exposed to intragastric infusions of ethanol for 6 days. There are consistent findings that during withdrawal, NMDA-Rs are over-activated pathologically by increased glutamate release and that this effect is accentuated by the interaction of polyamines acting via the 2B subunit.

AMPA receptor subunits GluR1, GluR2/3 and the kainate receptor subunits GluR5, GluR6 and KA2 are unaltered after 16 days of ethanol exposure. No adaptive changes of NMDA-R subunits are found in a recent study [6]. Therefore maladaptive changes in brain ionotropic glutamate receptor levels do not underlie, in all cases, the neurobiological consequences of chronic ethanol exposure.

Opioid Peptides

$\blacktriangleright$ Opioid systems in the brain are important for the reinforcing effects of ethanol. Selective μ -opioid receptor antagonists reliably decrease ethanol drinking in rats.

Chronic free-choice ethanol consumption causes increased β -endorphin immunoreactivity in the hypothalamus and septum of alcohol-preferring mice (C57BL/6), and Met-enkephalin in the nucleus accumbens of ethanol-preferring AA rats. Continuous-access ethanol consumption caused a significant decrease in preproenkephalin mRNA expression in the nucleus accumbens and olfactory tubercle and a significant increase in mRNA in nuclei of the amygdala of fawn-hooded rats. Ethanol consumption had no significant effect on preprodynorphin mRNA. Thus, ethanol seems to negatively regulate enkephalin expression *in vivo*. The increase of preproenkephalin mRNA in the amygdala may be caused by the facilitating effect of ethanol on gabaergic neurons.

Cyclic AMP (►Cyclic Adenonine Monophosphate)

Acutely, ethanol has been shown to potentiate G_{S} -stimulated cAMP accumulation. Conversely, brain tissue and cell culture treated chronically with ethanol have decreased levels of adenylyl cyclase activity [5]. Tolerance to chronic ethanol is accompanied by a fall in the levels of G_{aS} -proteins and G_{aS} -mRNA in NG108-15 neuroblastomaxglioma cells and an increase in G_{ai2} -proteins, in blood platelets from alcoholics up to 6 months after ethanol withdrawal [3]. However, the contention that an increased level of G_{ai} and a reduced level of G_{aS12} , respectively, are the cause of ethanol-induced tolerance was disputed in recent studies. Others have demonstrated a reduced catalytic activity of the ►adenylyl cyclase after 4 weeks ethanol in most but not all brain regions. The changes were not reflected by altered levels of the enzyme [3]. Among the nine isoforms of adenylylcyclase, the type VII was activated by acute ethanol (50 mM) and prostaglandin E_1 (10 μM) two- to threefold greater than that seen with the other tested adenylyl cyclases. PKC_{δ} is involved in ethanol modulation of AC activity. Ethanol could promote a conformational change in AC that provides or enhances availability of a site(s) for PKC_{δ} -mediated phosphorylation, or ethanol could promote the association of AC with PKC_{δ} within a transducisome complex. In the presence of ethanol the more phosphorylated form of AC7 becomes more sensitive to activated G_{sa} . The increased levels of cAMP during such a signaling process will produce a greater effect of PKA and a greater modification of downstream effectors dependent on cAMP signaling [5].

Clinical Use (Including Side Effects)

No clinical use, used in some medicinal drugs to solubilize active compounds.

- Drug Addiction/Dependence
- GABAergic System
- Ionotropic Glutamate Receptors
- Opioid System

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Ethyl Alcohol

►Ethanol

Euglycaemia

Euglycaemia (normoglycaemia) is a blood glucose concentration within the normal range, e.g. fasting blood glucose 3.5–6.5 mmol/l; postprandial blood glucose 5–11 mmol/l (reference ranges vary between laboratories).

- Diabetes Mellitus
- Insulin Receptor
- Oral Antidiabetic Drugs

Eukaryotic Expression Cassette

The eukaryotic expression cassette is the part of an expression vector that enables production of a protein in a eukaryotic cell. The cassette consists of a eukaryotic promoter for mRNA transcription, the gene and an mRNA termination and processing signal (Poly-A signal).

►DNA Vaccination and Genetic Vaccination

Excitability

Excitability refers to the capacity of nerves and other tissues (e.g. cardiac), as well as individual cells, to generate and sometimes propagate action potentials, signals that serve to control intracellular processes, such as muscle contraction or hormone secretion, and to allow for long- and short-distance communication within the organism. Examples of excitable cells and tissues include neurons, muscle and endocrine tissues. Examples of nonexcitable cells and tissues include blood cells, most epithelial and connective tissues.

- ▶ Voltage-dependent Na^+ Channels
- ▶ Nicotinic Receptors
- ▶ Ionotropic Glutamate Receptors
- ▶ Voltage-gated K^+ Channels
- ▶ Inward Rectifier K^+ Channels

Excitation–contraction Coupling

Excitation–contraction coupling (EC coupling) is the mechanism underlying transformation of the electrical event (action potential) in the sarcolemma into the mechanical event (muscle contraction) which happens all over the muscle. In other words, it is the mechanism governing the way in which the action potential induces the increase in the cytoplasmic Ca^{2+} which enables the activation of myofibrils.

- ▶ Ryanodine Receptor

Excitatory Amino Acids

- ▶ Glutamate

Excitotoxicity

Excitotoxicity is the over-activity of the glutamatergic system responsible for the large number of dead neurons observed after ischemia (stroke) or epileptic seizures. This neuronal death is due to an over-excitation of the neurons and the massive Ca^{2+} entry

resulting from the depolarization. Because of their large Ca^{2+} permeability, the NMDA iGlu receptors play a major role in the excitotoxic effect of glutamate. Excitotoxicity also may have a part in the pathogenesis of neurodegenerative disorders such as Huntington's disease and amyotrophic lateral sclerosis (ALS).

- ▶ Metabotropic Glutamate Receptors
- ▶ Neurotransmitter Transporters

Exenatide

Synthetic peptide which mimics the effect of glucagons-like peptide 1 (GLP-1) in stimulating glucose-dependent insulin secretion from pancreatic β -cells and inhibiting glucagon secretion from α -cells. Exenatide improves glycaemic control in type 2 diabetics and causes a significant reduction of body weight.

- ▶ Diabetes Mellitus

Exocytosis

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Synonyms

Constitutive secretion; Regulated secretion; Neuronal secretion

Definition

Cellular secretion from macromolecules mediated by fusion of vesicles with the plasma membrane

Basic Mechanisms

Constitutive and Regulated Exocytosis

During exocytosis, intracellular vesicles fuse with the plasmalemma. As a consequence, the vesicle components are incorporated into the plasma membrane and the vesicle content is released into the extracellular space. We distinguish constitutive and regulated exocytosis.

Constitutive exocytosis/secretion takes place in all eukaryotic cells and is essential for cell viability and growth. Trafficking vesicles destined for constitutive exocytosis originate from the trans-Golgi-network and contain secretory macromolecules derived from the

biosynthetic pathway. They are transported along microtubules to the cell surface and fuse with the plasma membrane. Constitutive secretion leads to the continuous release of secretory products and to the incorporation of membrane constituents into the plasmalemma. Hence, constitutive cargo is not stored within the cell. Excess plasma membrane is retrieved by endocytosis. The balance between exocytosis and endocytosis ensures ordered cell growth during proliferation and maintains a constant surface area in nondividing cells. Proteins secreted by constitutive exocytosis include immunoglobulins, serum and milk proteins, and proteoglycans. In addition to vesicles derived from the biosynthetic route, recycling vesicles derived from endocytic precursor organelles (mostly endosomes) also fuse constitutively with the plasma membrane, thus returning endocytosed membrane constituents to the surface.

Regulated exocytosis differs from constitutive exocytosis, in that secretion-ready vesicles are stored in the cytoplasm, often in large numbers, and that they require a stimulus for fusion with the plasma membrane. Regulated secretory cells include, for instance, exocrine and endocrine cells, mast cells, platelets, large granular lymphocytes, neutrophils, and neurons. Depending on the cell type, exocytosis can be triggered by a variety of physiological stimuli that exert their action by means of receptor activation or electrical excitation. Release kinetics range from milliseconds in neurons to many minutes in exocrine and certain endocrine glands. All stimuli ultimately cause the transient rise of an intracellular second messenger, in most cases calcium. Calcium either directly activates exocytosis or it operates in conjunction with cAMP and protein kinases that potentiate the effect of calcium. In some systems, calcium ions are only permissive, and the triggering stimulus involves other second messengers and signaling cascades. In electrically excitable cells, the intracellular calcium concentration rises after depolarization by calcium entry through voltage-gated calcium channels. In cells lacking regulated calcium influx pathways, hormones, acting mostly through G-protein-coupled receptors, cause the release of calcium from intracellular stores. Regulated exocytosis also differs from constitutive exocytosis in that secretory products are often packaged at high concentrations within the secretory vesicles. Thus secretory cells are capable of rapidly discharging large amounts of secretory products in a short time without the need for concomitant product biosynthesis.

In addition to secretory cells, many non-secretory cells are capable of regulating exocytotic fusion of transport vesicles that are derived from endosomal precursors. For instance, vesicles enriched in plasma membrane transport proteins are incorporated in a regulated manner in order to alter metabolite fluxes. Examples include the glucose transporter GLUT-4 in muscle and fat tissues, a key element in the control of

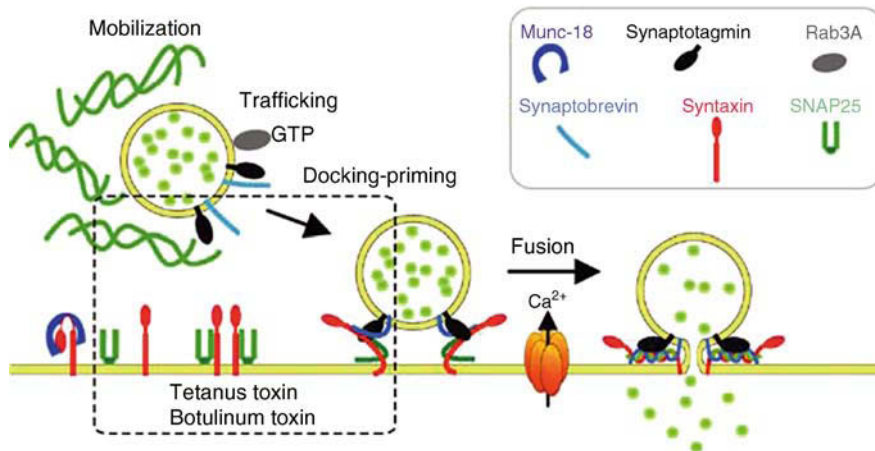
serum glucose levels, and the vacuolar proton ATPase and aquaporins in the kidney that are essential for pH and water homeostasis. Last not least, regulated fusion of intracellular vesicles is used by many cells to repair tears in the plasma membrane.

Molecular Mechanisms of Exocytosis

Before exocytosis, the vesicle first moves from the cytoplasm to the plasmalemma. Second, the vesicle becomes attached to the plasma membrane, a process often referred to as docking. Third, activation involving metabolic energy, also referred to as priming, is required to achieve fusion competence. Finally, the vesicle and plasma membranes merge (Fig. 1). Each of these steps involves a multitude of proteins that operate by complex protein–protein and protein–phospholipid interaction networks. Although we are still far from a comprehensive molecular description, it is becoming clear that these steps are mediated by sets of common proteins that belong to evolutionary conserved protein families. While these proteins appear to operate in all intracellular fusion events (probably with the exception of mitochondria and peroxisomes), they are controlled by additional factors specific for a given fusion event or a given cell type.

Transport of vesicles occurs along microtubular tracks with the aid of kinesin motor proteins. In contrast, interactions with the actin-based microfilament network are thought to regulate the availability of vesicles for fusion, and they are also involved in certain types of vesicle movement. For instance, in neurons synaptic vesicles are thought to be attached to the actin cytoskeleton by crossbridges of synapsins that bind both to synaptic vesicles and to actin. Upon activation, synapsins are phosphorylated by calcium- and cAMP-dependent protein kinases, resulting in a weakening of binding affinities and the release of the vesicles from the cytoskeleton.

The initial contact between the vesicle and the plasma membrane is mediated by protein complexes that appear to be essential for ensuring that only appropriate membranes fuse. While more is known about the proteins involved in the “homotypic” docking of intracellular transport vesicles than in docking of vesicles to the plasma membrane, similar mechanisms may be involved. A key role has been assigned to Rab proteins, a family of Ras-related small GTPases. Apparently, each fusion step is controlled by a specific family member that resides on the vesicle membrane and appears to play a key role in recruiting docking proteins to the vesicle surface. Like ras, Rabs operate as molecular switches that are active in the GTP-form and inactive in the GDP-form. Active Rabs recruit a variety of structurally diverse effector proteins to the vesicle that may then bridge the membranes destined to fuse. Protein recruitment may also be assisted



Exocytosis. Figure 1 Model for protein-mediated membrane fusion in neuronal exocytosis. Syntaxin and SNAP-25 form a complex on the plasma membrane that interacts with synaptobrevin on the synaptic vesicle membrane. The assembly of the resulting ternary complex forces the opposing membranes into close apposition. Such complex assembly may be enough to induce constitutive exocytosis. For neuronal exocytosis, fusion requires calcium that enters the cell via voltage-gated calcium channels. Triggering of fusion involves probably Ca^{2+} -induced interactions implicating synaptotagmin. A network of cytoskeletal elements controls the availability of secretory vesicles at the plasma membrane, and targeting of synaptic vesicles to docking sites may be carried out by Rab-GTPases. Tetanus and Botulinum toxins block exocytosis by specifically cleaving the neuronal SNAREs.

by the phospholipid phosphatidylinositol biphosphate (PIP₂). Several Rab effectors possess characteristic PIP₂-binding domains, and it is known that interference with the metabolism of PIP₂ inhibits exocytosis in some systems.

Membrane fusion itself is probably the best understood step in the sequence of events leading to exocytosis, although many questions remain. Essential for fusion are the **SNAREs**, a superfamily of small membrane proteins. Appropriate sets of three to four SNAREs spontaneously assemble from unstructured precursors into tight α -helical complexes that need metabolic energy for disassembly, and the assistance of the chaperone-like ATPase **NSF**, with additional cofactors for disassembly. Different sets of SNAREs are required for the various intracellular fusion steps. When appropriate SNAREs on the membranes destined to fuse contact each other, assembly is thought to pull the opposing membranes tightly together, a process that may suffice to initiate membrane fusion (Fig. 1). After fusion, the spent SNARE complexes are regenerated by ATP and NSF.

While the basic features of SNARE assembly and disassembly provide a convenient framework for explaining how membrane fusion works, both the regulation of SNAREs and the molecular details of fusion are not well understood. Most is known about the neuronal SNAREs that mediate regulated membrane fusion of synaptic vesicles and of secretory granules in neuroendocrine cells. They include synaptobrevin2, localized to the synaptic vesicle, and SNAP25 (**SNAPs**) and syntaxin1A, both of which are localized to the plasma

membrane. Several proteins are known that bind to these proteins and thus may regulate their activity. They include Munc-18, a syntaxin-binding protein, complexins that bind only to the fully assembled SNARE complex, and tomosyn which possesses a SNARE-like domain and competes with synaptobrevin. Genetic and physiological studies assign essential roles to these proteins in exocytosis, although their mechanism of action remains to be established.

While the steps described above are common to all exocytotic events, regulated secretion is distinguished by an additional layer of control proteins. Principally, each of the steps may be subject to regulation and thus controls the overall rate of exocytosis, and there appears to be a large variety of mechanisms that is reflected in a kinetic range covering several orders of magnitude. Even for the universal second messenger calcium there seems to be no universal mechanism of action. In chromaffin cells and possibly also in neurons, calcium controls the rate of several distinct and consecutive steps, the last being directly linked to membrane fusion. A prime candidate for the neuronal calcium receptor in the last step is **synaptotagmin**, a transmembrane protein of synaptic and secretory vesicles. Synaptotagmin possesses two calcium-binding modules (referred to as C2 domains) that interact in a calcium-dependent manner both with phospholipids and with SNAREs. Deletion of synaptotagmin largely abolishes calcium-dependent exocytosis, whereas exocytosis can still be elicited by calcium-independent pathways. In other cells, however, the control of the exocytotic rate appears

to occur at an earlier stage, e.g., by regulating vesicle availability through cytoskeletal interactions. Identifying and characterizing such control mechanisms remains one of the most urgent tasks for future research.

While recent attention has been largely on proteins, it should be borne in mind that membrane fusion ultimately involves the merger of phospholipid bilayers. However, little is known about the specific membrane lipid requirements. When membranes fuse, energetically unfavorable transition states are generated that may require specific lipids and lipid domains for stabilization. Although there is some evidence for a specific influence of lipids on exocytosis, it is still unclear whether specific lipid metabolites are needed or even generated at the site of membrane merger.

Pharmacological Intervention

Exocytosis represents the final step in a multistep pathway involving vesicle formation and storage, transport to the plasma membrane by microtubule dependent transport, possibly involving additional cytoskeletal elements (e.g., the actin-myosin system), vesicle attachment to the plasma membrane, vesicle activation ("priming"), and finally membrane fusion. Each of these steps is probably regulated, but the details of such regulation have only been worked out in a few cases. Exocytosis can be regulated by many membrane receptors, dependent on the cell type, via second messengers or by electrical activity. Accordingly, regulated exocytosis can be controlled by appropriate receptor agonists and antagonists, by drugs influencing second messenger levels (e.g., Ca-channel blockers) or by reagents interfering with the cytoskeleton. However, most of the second messenger targets in exocytosis remain to be identified, and it is conceivable that they may emerge as attractive drug targets. For instance, there is evidence that protein phosphorylation by protein kinases potentiates calcium-dependent exocytosis and in some cases suffices to induce exocytosis by itself, but it is not known which of the phosphorylated proteins are rate-limiting. Furthermore, the release of calcium from internal stores by second messengers may be an interesting point to control exocytosis by externally applied drugs. For example, hormones from pituitary gonadotropes are secreted in response to gonadotropin-releasing hormones. The gonadotropin-releasing hormone receptor couples to a G-protein, which activates the phospholipase C cascade with production of inositol trisphosphate and oscillatory release of calcium from intracellular stores. Hormone secretion may be controlled by interfering specifically with one of the steps in the cascade. Several biological toxins are known to directly affect exocytosis. Best characterized are the botulin and tetanus neurotoxins, proteinaceous AB-toxins with a heavy chain mediating cell entry and a light chain that carries the catalytic

activity. All light chains are proteases that cleave one of the three neuronal SNAREs and some of their close relatives. As a result, neuronal exocytosis is irreversibly inhibited. While extremely toxic upon systemic application, local application of botulinum neurotoxin A has become the treatment of choice for blepharospasm, hemifacial spasm, cervical dystonia, and laryngeal dystonia. The toxin also alleviates pain and may be used in therapeutic trials for prediction of the response to surgical elongation. New toxin serotypes are now being tested. There are also cosmetic uses of Botulinum neurotoxin A. Local injections are used to diminish the undesirably negative and expressive wrinkles of the face by producing a reversible weakness of the hyperfunctional mimetic muscles of facial expression. In addition, several animal toxins block neurotransmission but the mechanism is different. These toxins cause massive exocytosis until the synaptic vesicle pool is exhausted. Best studied among these toxins is α -latrotoxin, the active ingredient of black widow spider venom, and probably also some snake toxins (e.g., crotoxin, taipoxin). Their mechanism of action, however, is not understood.

- Synaptic Transmission
- Small GTPases
- Intracellular Transport
- Bacterial Toxins

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Exogenous Pyrogens

Extraneous live microorganisms or their products or certain inanimate substances recognized as foreign and potentially deleterious by the afflicted host that promptly evoke fever. They do this, in most cases, by inducing endogenous pyrogens, obligatory inherent mediators of the febrile response. Endotoxic bacterial lipopolysaccharide is an exogenous, bacterial pyrogen.

- Fever
- Endogenous Antipyresis
- Endotoxin

Exon

A length of DNA in a gene that is transcribed into mRNA and translated into the final protein product.

► Intron

Exopeptidase

A peptidase that acts only at the amino- or carboxy-termini of proteins or peptides.

► Non-viral Peptidases

Exportins

Exportins are transport receptors at the nuclear pore complex needed for the selective export of proteins from the nucleus into the cytoplasm. They recognize nuclear export signal sequences of cargo proteins.

► Small GTPases

Extended Amygdala

The shell of the nucleus accumbens, the bed nucleus of the stria terminalis, and the central nucleus of the amygdala, together referred to as the extended amygdala, may play a role in drug addiction.

► Drug Addiction/Dependence

Extracellular Calcium-sensing Receptor (CaSR)

► Ca^{2+} -sensing Receptor

Extracellular Matrix (ECM)

In biology, extracellular matrix (ECM) is the extracellular part of animal tissue that usually provides structural support to the cells in addition to performing various other important functions. ECM is the defining feature of connective tissue in animals. ECM includes the interstitial matrix and the basement membrane.

► Matrix Metalloproteinases

Extrapyramidal Side Effects

Extrapyramidal side effects (EPS) are adverse effects of dopamine D_2 -receptor antagonists (e.g. metoclopramide) acting on the nigrostriatal system. EPS include parkinsonism (stiffness, slow movements, stooped posture, and tremor of extremities), akathisia (an inner sense of restlessness or need to move), and dystonia (spasm of a muscle group, most commonly involving the neck, extraocular muscles, and the tongue). Dose adjustment is often sufficient in managing these side effects, though use of other medications such as beta blockers (e.g. propranolol for akathisia), anticholinergic medications (e.g. benztropine for dystonia and parkinsonism), and amantadine (for parkinsonism), may sometimes be necessary. The dopamine D_2 -receptor antagonist domperidone does not readily penetrate the blood brain barrier. It is therefore less prone to produce EPS.

► Antipsychotic Drugs

► Dopamine System

► Emesis

Extrasynaptic Receptors

Populations of receptors that are excluded from synaptic junctions. These may be distributed over neuronal cell bodies or located around but not directly beneath synapses (perisynaptic). Some receptors have become specialised to serve an extrasynaptic function producing a tonic level of activity in response to ambient levels of neurotransmitter. This tonic current can be used to maintain homeostatic control over neuronal excitation.

F-actin

F-actin (also called microfilament or actin filament) is a double-stranded, right-handed helix with 14 actin molecules per strand and turn. F-actin has a diameter of 8 nM and is polarized with a pointed (minus) and a barbed (plus) end.

► Cytoskeleton

Fab Fragments

Fab fragments are variable (specific) regions of antibodies.

► Humanized Monoclonal Antibodies

Factor IIa

- Thrombin
- Coagulation/Thrombosis
- Anticoagulants

FAD

Flavin Adenine Dinucleotide

- Flavin Adenine Dinucleotide
- Vitamin B₂ (Riboflavin)
- Monoamine Oxidases and their Inhibitors

Familial Alzheimer's Disease

This type of disease occurs in families and begins unusually at early age (i.e., onset below the age of 60). Approximately 10% of Alzheimer's disease are familial and are inherited in an autosomal dominant manner with high penetrance. Deterministic genes directly cause the disease. Mutations in three different genes encoding for the amyloid precursor protein (APP) and the presenilins 1 and 2 (PS1 and PS2) have been identified to be responsible for early-onset familial Alzheimer's disease.

In addition, other genes (risk genes) remain to be discovered. Risk genes, such as the apolipoprotein E-e4 (APOE-e4) gene, increase the likelihood of developing the disorder.

► Alzheimer's Disease

Familial Hypocalciuric Hypercalcemia (FHH)

A form of PTH-dependent hypercalcemia that results from the presence of one or two CaR alleles bearing inactivating mutations.

► Ca²⁺-Sensing Receptor (CaR)

Fanconi-Bickel Syndrome

Fanconi-Bickel syndrome (FBS) is a rare type of glycogen storage disease which is caused by homozygous or compound heterozygous mutations within the *SLC2A2* gene encoding GLUT2. More than 100

patients have been reported in the literature. Most patients have the typical combination of clinical symptoms: hepatomegaly secondary to glycogen accumulation, glucose and galactose intolerance, fasting hypoglycemia, a characteristic tubular nephropathy, and severely stunted growth. A total of 34 different *SLC2A2* mutations have been identified with none of them being particularly frequent. No specific therapy is available for FBS patients. Symptomatic treatment is directed towards a stabilization of glucose homeostasis and compensation for renal losses of various solutes.

► Glucose Transporters

Farnesyl Transferase Inhibitors (FTIs)

► Lipid Modifications

FAS Ligand (FasL)

The Fas ligand or FasL is a type II transmembrane protein that belongs to the tumor necrosis factor (TNF) family. The binding of Fas ligand with its receptor induces apoptosis.

- Matrix Metalloproteinases
- Tumor Necrosis Factor
- Apoptosis

FAT10

F-adjacent Transcript-10 (FAT10) is composed of two ubiquitin-like domains and capable to mark conjugated proteins for proteasomal degradation independent of ubiquitin. FAT10 is inducible by IFN- γ and TNF and induces apoptosis when over expressed.

► Ubiquitin/Proteasome

Fatty Acid Transporters

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Acronym

FATP

Synonyms

Fatty acid transport protein paralogues 1–6; FATP1–6; Gene symbols SLC27A1–6; Solute carrier family 27A; Very long-chain acyl-CoA synthetase; VLCS

Definition

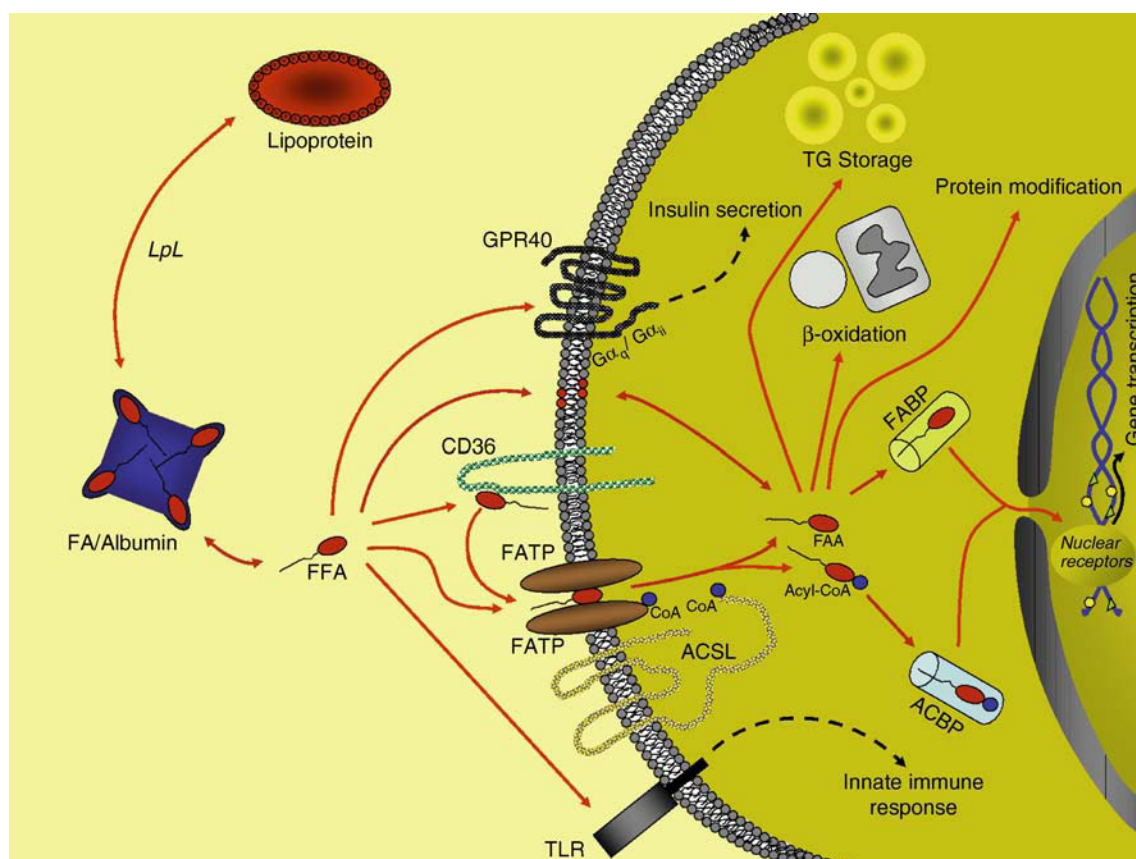
Fatty acid transport proteins (FATPs) are an evolutionary conserved family of integral membrane proteins found at the plasma membrane and on internal membranes. FATPs facilitate the unidirectional uptake and/or intracellular activation of unesterified long-chain and very ► [long-chain fatty acids](#) (LCFAs) into a variety of lipid-metabolizing cells and tissues.

Basic Mechanisms

Introduction

LCFAs are vital components of our diet and contribute to cellular processes including metabolic energy generation and storage, plasma membrane synthesis, and protein anchoring. While in some tissues and cell types LCFAs can signal through membrane receptors, they typically have to first cross the plasma membrane to elicit cellular responses. In general, the uptake of fatty acids from the circulation into cells includes the sequence of (i) localized generation of free fatty acids through hydrolysis of triglycerides (TGs) from lipoproteins by lipases inside the endothelial lumen and rapid binding of fatty acids to albumin, (ii) fatty acid dissociation from albumin followed by passive or active transport across the plasma membrane, and (iii) their association with intracellular binding proteins and subsequent participation in metabolic and signaling processes ([Fig. 1](#)).

Uptake of LCFAs across the lipid-bilayer of most mammalian cells occurs through both a passive diffusion of LCFAs and a protein-mediated LCFA uptake mechanism. At physiological LCFA concentrations (7.5 nM) the protein-mediated, saturable, substrate-specific, and hormonally regulated mechanism of fatty acids accounts for the majority (>90%) of fatty acid uptake by tissues with high LCFA metabolism and storage such as skeletal muscle, adipose tissue, liver,



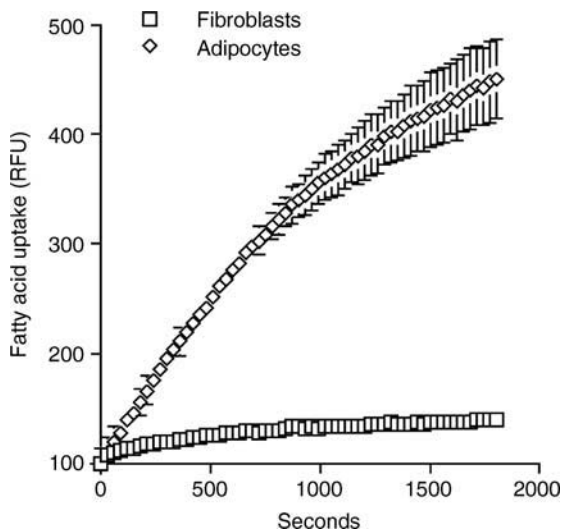
Fatty Acid Transporters. Figure 1 Free fatty acid uptake and action in mammalian cells. Serum free fatty acids (FFA) are generated from lipoproteins by the action of endothelial lipoprotein lipase (*LpL*). At physiological conditions, the majority of FFAs is bound to albumin while the concentration of unbound FFAs in this equilibrium is low. Plasma membrane traversing of FFAs into the cell under these conditions occurs mainly by a protein-mediated mechanism, either by interaction of the FFAs directly with FATP complexes or by a preceding binding to cell-surface proteins, such as CD36, which subsequently hands the FFAs on to the FATPs. On the cytosolic site, FFAs are quickly activated and coupled to coenzyme A (CoA) by the catalysis of long-chain fatty acyl-CoA synthetases (ACSLs) or the by FATPs itself. Fatty acid binding proteins (FABPs) or acyl-CoA binding proteins (ACBPs) facilitate an intracellular unloading of the transporters and the synthetases and can also function as an intracellular fatty acid buffer. In the cell, FFAs can act at different subcellular localizations and have functions in energy generation and storage, membrane synthesis, protein modification, and activation of nuclear transcription factors. In addition to acting intracellularly, unbound FFAs can also signal extracellularly in certain cell types (e.g., by stimulating the G-protein coupled receptor GPR40 in β -cells to induce insulin secretion, or by activating toll-like receptors – TLRs) to initiate the innate immune response).

and heart. The relative contribution of passive diffusion is thought to be higher in cell types that lack fatty acid transporters such as fibroblasts (see Fig. 2) and may be elevated as a result of super-physiological fatty acid concentrations. While several fatty acid handling proteins have been identified (see Fig. 1), recent in vivo studies have particularly highlighted the contribution of FATPs to LCFA uptake, lipid metabolism, and lipid-associated disorders [1].

The Family of FATPs

In humans and mice, the FATP family is comprised of six structurally related members (FATP1–6; 33–57.1%

identity in mice) that are found in all fatty acid-utilizing tissues of the body. While substrate preferences are comparable among the different FATPs paralogues, they differ widely in their tissue expression pattern [2]. FATP1 was the first FATP to be identified and is highly expressed in adipose tissue, skeletal muscle and, to a lesser extent, in heart. FATP2 is predominantly expressed in liver and kidney cortex. FATP3 shows a broader expression pattern with high mRNA and protein levels in lung. FATP4 is the only FATP expressed in small intestine and is localized to the apical brush border of the epithelial cells, where it is implicated in the absorption of dietary lipids. FATP4



Fatty Acid Transporters. Figure 2 Quencher-based real-time fatty acid uptake assay with a fluorescently labeled FFA analogue (C1-Bodipy-C12). Predominantly protein-mediated fatty acid uptake by 3T3-L1 adipocytes (diamonds) was compared with diffusion-driven uptake by fibroblasts (squares) using the QBT Fatty Acid Uptake reagent (Molecular Devices Corp., CA, USA), which contains C1-Bodipy-C12 as substrate in conjunction with a cell impermeable quencher. Uptake kinetics was recorded using a Gemini fluorescence plate reader. Error bars indicate the standard deviations from 12 independent wells. RFU: relative fluorescence units.

is also expressed to a lesser extent in other tissues including adipose tissue, liver, skin, and heart. Furthermore, FATP4 together with FATP1 are the predominant FATP paralogues in brain. FATP5 is expressed solely in liver. Confocal immunofluorescent microscopy with isolated primary hepatocytes demonstrated that FATP5 is localized to the plasma membrane of these cells, which was confirmed by immunoelectron microscopy of liver sections, showing a predominant localization of FATP5 protein to basal microvilli in the space of Disse. FATP6 is expressed specifically in heart where it is the predominant FATP paralogue. Immunofluorescence microscopy studies of FATP6 in primate and murine hearts have shown that the protein is exclusively located on the sarcolemma, where it is restricted to areas of the plasma membrane juxtaposed to small blood vessels [1]. In addition to vertebrates, FATP orthologs have been identified in invertebrate, fungi, and prokaryote genomes such as *Caenorhabditis elegans*, *Saccharomyces cerevisiae*, and *Mycobacterium tuberculosis*.

FATP Structure and Mechanism of Transport

Due to the high overall sequence similarity, the presumed secondary structure of all FATP members is assumed to be similar, with an extracellular N- and an intracellular

C-terminus. Because of the hydrophobic nature of a protein that strongly interacts with fatty acids, the exact topology of the FATPs is difficult to predict. Studies with FATP1 revealed at least one α -helical transmembrane domain and several membrane-associated domains [3]. However, FATPs do not show any obvious similarities to other transporter families, e.g., the polytopic membrane transporters for hydrophilic substrates such as members of the GLUT family, or transporters of amphipathic and hydrophobic substrates, e.g., bile and cholesterol transporters. It has been demonstrated that FATP1 forms homodimers, and possibly higher-order complexes, most likely by interactions in the cytoplasmic loop. Coexpression experiments of nonfunctional FATP1 mutants with wild-type FATP1 revealed that oligomerization is required for their transport function. A 311-amino acid sequence motif is conserved in all mammalian FATP family members and is essential for the function of these proteins. An AMP-binding sequence (IYTSGTTGXPK), likely facing the cytosol, is found at the beginning of this sequence motif. This 11-amino acid motif is conserved in a number of proteins that either bind ATP or catalyze reactions that proceed through adenylated intermediates.

In vitro and ex vivo studies have shown that FATPs transport LCFAs and very long-chain fatty acids (VLCFAs) but no medium-chain fatty acids, fatty acid esters, or lipid-soluble vitamins [4]. LCFA transport is inhibited by prior protease treatment. Synthetic substrates for FATPs include 14 C-labeled fatty acids and the fluorescently labeled fatty acid analogue C1-BODIPY-C12. Using the latter substrate, differences in fatty acid uptake kinetics between FATP expressing 3T3 L1 adipocytes and 3T3 L1 fibroblasts, which are devoid of FATPs, can be readily appreciated (Fig. 2).

It has been shown that FATP1, -2, -3, -4, and -5 can also catalyze the formation of CoA thioesters of hydrophobic substrates such as VLCFAs and unconjugated bile acids. However, it is still an ongoing debate whether FATPs are (i) solely transmembrane transport proteins mediating LCFA uptake, possibly in close association with other proteins such as long-chain acyl-CoA synthetases (ACSLs), or (ii) are themselves membrane-bound long-chain and very long-chain ACSLs that trap LCFAs inside the cell following fatty acid diffusion across the plasma membrane, or (iii) combine the transport with acyl-CoA synthetase activity for optimal uptake, or (iv) are multifunctional proteins that mediate LCFA uptake independently of their esterification activities. Clearly, further investigations of the enzymatic and transport activities of FATPs are needed to resolve these important questions. Extrapolating from our current knowledge, it is likely that in vivo several fatty acid handling proteins such as fatty acid translocase (FAT/CD36), long-chain ACSLs, fatty acid binding proteins (FABPs), and acyl-CoA

binding proteins (ACBPs) interact with FATPs to facilitate efficient uptake of fatty acids (Fig. 1). In accordance with this hypothesis, FATP1 has been shown to associate with ACSL1 [5], suggesting that both proteins are essential for FFA uptake. As a model for LCFA uptake we have suggested the following mechanism [3]. In addition to a small, diffusional component, LCFAs are either directly transported by FATP complexes across the plasma membrane or, alternatively are first accumulated on the plasma membrane by binding to CD36, which subsequently hands on the fatty acids to FATPs. Within the cells, LCFAs are rapidly activated and metabolized by ACSLs or FATPs. Subsequent binding of fatty acids to intracellular LCFAs handling proteins facilitates the unloading of transporters and synthetases and acts as an intracellular fatty acid buffer.

FATP Null Mutants and FATP Polymorphisms

►**Knockout mice** have been reported for several FATPs [1]. As ►**insulin desensitization** has been closely linked to excessive fatty acid uptake and intracellular diacylglycerol and TG accumulation, these animal models were particularly evaluated in the context of protection from diet-induced type 2 diabetes (►**Type 2 Diabetes Mellitus** (T2DM)). In addition, studies on human subjects have also established genetic links between ►**polymorphisms** in FATP genes and metabolic alterations [1].

In hyperinsulinemic-hyperglycemic clamp studies, FATP1 KO mice were protected from the insulin-desensitizing effects of lipid injections. FATP1 KO mice were completely resistant to long-term diet-induced ►**obesity**, insulin-desensitization, and other parameters of the metabolic syndrome. Loss of FATP1 function reduced muscle TG content and prevented a lipid bolus induced reduction of IRS-1 tyrosine phosphorylation and PI-3 kinase association. FATP1 is also expressed on the plasma membrane of brown adipose tissue (BAT) and FATP1 KO mice showed reduced basal fatty acid uptake and displayed smaller lipid droplets in BAT. As a consequence FATP1 KO mice failed to upregulate fatty acid uptake and to defend their core body temperature following cold exposure suggesting that FATP1 is required for thermogenesis [6].

As yet, no human diseases have been identified as a result of FATP1 mutations. However, genetic polymorphisms in the human FATP1 gene have been linked to dyslipidemia. An A/G exchange at position +48 in intron 8 of the FATP1 gene has been shown to result in increased TG concentrations in female but not in male subjects. In a second study, the same polymorphism was linked to increased postprandial TG concentrations and smaller low density lipoprotein (LDL) particles. To date, it is still unknown if this polymorphism is associated with altered levels of FATP1 expression and/or function.

A FATP2 KO mouse has been generated and investigated in the context of the neurodegenerative endocrine disorder X-linked adrenoleukodystrophy. However, no association between FATP2 function and X-linked adrenoleukodystrophy was found. FATP2 KO mice exhibited a decreased peroxisomal very long-chain acyl-CoA synthetase activity and decreased peroxisomal VLCFA β -oxidation in liver and kidney. However, no VLCFA accumulation in either of these organs was observed. The consequences of FATP2 loss for hepatic and renal LCFA uptake are presently unknown.

To date, four studies on murine FATP4 deletions have been published. Both the introduction of a premature stop codon in exon 3 due to a spontaneous mutation as well as the deletion of exon 3 by gene manipulation resulted in an early neonatal lethality due to symptoms strikingly similar to restrictive dermopathy, a rare human genetic disorder. Neonate mice exhibited thickened, tight skin, and a disrupted epidermal barrier as well as facial deformations and breathing difficulties. Additional analyses of the genetically engineered mice demonstrated reduced esterification activities for C24:0, but not C16:0 or C18:1 (very) long-chain fatty acids in dermal and intestinal lysates from FATP4 null mice. No effects on LCFA uptake have been examined in these two studies. A third study described a deletion of exons 1 and 2 of the murine FATP4 gene resulting in embryonic lethality occurring before day 9.5 of gestation. This phenotype was ascribed to the absence of FATP4 in the yolk sac where it is normally expressed by the cells of the extra-embryonic endoderm, likely resulting in an impaired absorption of maternal lipids by the embryo during early embryogenesis. Isolated primary enterocytes from heterozygote mice had a 48% reduction in FATP4 expression and a 40% reduction in LCFA uptake. However, no malabsorption of lipids was detected in vivo. A fourth report describing an epidermal-specific conditional FATP4 KO mouse showed that these mice develop a hyperkeratosis with a disturbed epidermal barrier suggesting that epidermal FATP4 is essential for the maintenance of a normal skin structure and function. In humans, a polymorphism in exon 3 of the FATP4 gene leads to an amino acid exchange (G209S) that has been linked to decreased parameters for TG and insulin levels, body mass index (BMI), and systolic blood pressure.

FATP5 KO mice have been characterized in two studies focusing on the role of FATP5 in hepatic lipid and bile metabolism. LCFA uptake in primary hepatocytes isolated from FATP5 KO mice was reduced by 50% and hepatic lipid content in the KO mice was significantly reduced despite an increased fatty acid de novo biosynthesis. Detailed analysis of the hepatic lipidome of FATP5 KO mice revealed significant

quantitative and qualitative alterations among lipid classes. Similarly to FATP1 KO mice, homozygote deletion of FATP5 resulted in resistance to high-fat diet-induced weight gain and insulin-resistance. FATP5 KO mice displayed both decreased caloric intake and increased energy expenditure on this diet. How changes in hepatic lipid metabolism lead to altered feeding behavior is currently unknown. While the total bile pool in FATP5 KO mice was unchanged, a distinct shift from conjugated to unconjugated bile acids occurred as a result of FATP5 deletion. The remaining conjugated bile acids were exclusively derived from de novo synthesis, implying a role of FATP5 in the reconjugation of bile acids during enterohepatic recirculation.

Pharmacological Intervention

Insulin

Insulin stimulates FATP1-mediated fatty acid transport on the protein level but negatively regulates FATP1 mRNA transcription through a cis-acting insulin response promoter sequence, albeit no changes in FATP1 protein levels have been reported following insulin exposure. In basal adipose and muscle cells, most of the FATP1 protein is sequestered in an intracellular perinuclear compartment, where it colocalizes with the insulin-sensitive glucose transporter GLUT4. Insulin stimulation induces the translocation of FATP1-containing vesicles to the plasma membrane resulting in an increase in cellular LCFA uptake.

Phloretin

Phloretin inhibits FATP-mediated traversing of fatty acids across lipid bilayers. Phloretin is the aglycon of phlorizin and has been used to terminate the uptake of LCFAs and VLCFAs in timed in vitro uptake assays with cultured cells or in ex vivo uptake assays with isolated primary cells.

Thiazolidinediones

A peroxisome proliferator-activated receptor (PPAR) binding site was identified in the murine FATP1 promoter. Several reports have shown a positive regulation of mouse FATPs by ligands that activate PPAR- α , PPAR- γ , or PPAR- γ /RXR heterodimers.

TNF

Tumor necrosis factor alpha (TNF α) is a negative regulator of FATP expression and downregulates FATP mRNA and protein levels in several tissues.

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Favism

Favism is the haemolysis observed after eating Vicia fava. This reaction is observed in individuals with glucose-6-phosphate dehydrogenase deficiency. This common deficiency is also responsible for haemolysis in response to the antimalarial drug primaquine and others.

► Pharmacogenetics

Fc Receptor

Immune receptors that recognize the Fc (fragment, crystallizable) of antibodies when bound to pathogen, thereby exerting effector functions such as phagocytosis or antibody-dependent cell-mediated cytotoxicity.

► T Cell Receptors

Fever

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Synonyms

Pyrexia; (Hyperpyrexia)

Definition

The term *fever* specifically defines the elevation of body core temperature (T_c) that characteristically occurs in most animals in response to the invasion of their body by infectious organisms such as bacteria, viruses, and fungi. It is also a frequent reaction to nonmicrobial illnesses (e.g., autoimmune and neoplastic diseases), some host-derived substances (e.g., antigen–antibody complexes), as well as to certain synthetic products (e.g., antitumor agents, immunoadjuvants). The English word *fever* stems from the Latin word *febris*; pyrexia is a synonym derived from the Greek word *pyretos*. In humans, T_c s above 38°C are generally considered clinical fevers. Hyperpyrexia is a medical emergency defined as a T_c over 41.1°C.

Our knowledge of the underlying mechanism of fever derives largely from research using animals. Thus, generally in such studies, a fever-producing substance (►**exogenous pyrogen**) is administered as a bolus to conscious rodents. In most cases, this material is an extract of the outer wall of gram-negative bacteria (*bacterial endotoxic lipopolysaccharide*, LPS); a multiphasic rise in T_c is consequently generated that, due to its causative agent, is prototypic ►**endotoxic fever**. Importantly, however, this T_c rise is but one among an array of systemic, nonspecific, highly coordinated reactions typifying gram-negative bacteremia, all directed to combating the deleterious effects of the pathogenic compound. They are termed collectively the *acute-phase reaction* (APR), a pathognomonic collection of behavioral (Table 1) and physiological (Table 2) responses. Fever is the most manifest among these signs and, therefore, it is the hallmark of infection. Indeed, since the early 1900s, the measurement of body temperature has become a clinically routine practice in the detection of infectious disease.

Basic Mechanisms

The Febrile Course

T_c does not rise immediately following the entry into the body of an infectious pathogen. Rather, there is an

interval, from minutes to days, during which the various host-generated reactions that eventuate in the development of fever (and the other components of the APR) take place. This period is called the *latent* or *prodromal* period. Its duration depends on the nature of the invading microorganisms and/or its products, its route of entry, its amount, the site of its localization, certain physiological variables of the afflicted host, and other factors. It is succeeded by the *phase of rising T_c* or *febrigenesis*. The height, duration, and other characteristics of the ensuing fever are also largely dependent on the amount and type of the pathogen. They can, however, be influenced by various endogenous (e.g., state of hydration) and exogenous (e.g., climatic) factors; to wit, dehydration and high ambient temperature (T_a) exacerbate fever. The upper limit of fever in humans is ~40.5°C. When T_c reaches its maximum, it remains there for a period of time, termed the *stable* or *plateau phase* or *fastigium*. Its magnitude and duration are also related to the dose (severity of the infection) and type of the pathogen, and can also be modified by extraneous factors. Finally, the fever breaks (*crisis*) and T_c begins to decrease toward its normal level. This phase is variously called the *phase of falling T_c* , *febrilysis*, or *defervescence*.

Functionally, the onset of fever is mediated by an increase in metabolic heat production (*thermogenesis*) and cutaneous vasoconstriction (to reduce heat loss from the skin), and by cessation of sweating, if present. The

Fever. Table 2 Some characteristic physiological responses to infectious pathogens or their products (the “acute-phase reaction”)

Fever ↑
Slow-wave sleep ↑
Pituitary hormones↑↓ ACTH↑, PRL↑, GH↑, AVP↑, αMSH↑, βEndo↑, SRIF↑ LH↓, TSH↓
Plasma Fe↓, Zn↓, Cu↑
Erythropoiesis↓ (anemia)
Circulating neutrophils↑
Sympathetic nervous activity ↑
Acute-phase proteins↑↓ CRP↑, complement↑, PLA ₂ ↑, serum amyloid A↑, fibrinogen↑, α ₁ -acid glycoprotein↑, IL-1Ra↑, ceruloplasmin↑, α ₁ -antichymotrypsin↑, LBP↑ albumin↓, haptoglobin↓, IGF-1↓, transferrin↓, α ₂ -HS glycoprotein↓
Bone substance ↓
Muscle proteolysis↑
Pancreatic insulin↑, glucagon↑
Lipogenesis↑
Gluconeogenesis↓

Fever. Table 1 “Sickness behaviors” typically elicited by infectious pathogens or their products

Hyperalgesia, later hypoalgesia
Lethargy, somnolence
Anorexia, adipsia
Weakness, malaise
Decreased locomotor activity
Inability to concentrate
Loss of interest in usual activities, listlessness
Disappearance of body-care activities
Withdrawal

increased heat production is achieved by the most visible sign of fever production, shivering (*chills*); in neonates and cold-acclimated rodents, in which shivering thermogenesis is normally replaced by brown adipose tissue (BAT) nonshivering thermogenesis, the latter substitutes for shivering in the production of fever. In a thermally neutral environment ($\sim 22\text{--}24^\circ\text{C}$ for humans), the contribution of increased thermogenesis to the T_c rise is usually relatively brief; a greater role is played by the reduction in blood flow to the skin effected by cutaneous vasoconstriction. This is manifested by a cold and pale skin, provoking subjective sensations of cold that prompt the afflicted subject to actively (i.e., behaviorally) seek warmer surroundings. The thermoeffector responses evoked are thus analogous to those caused by acute cold exposure; but, since they occur in a warmer environment, the consequently narrower gradient between skin (T_{sk}) and ambient (T_a) temperatures results in less heat flowing from the skin to the environment and more, therefore, being retained in the body; hence, T_c rises. Fever developing in the cold, however, requires more intense heat production, whereas a decrease in heat loss (i.e., more generalized cutaneous vasoconstriction) may be sufficient in the heat. During the plateau phase of fever, the cutaneous vasculature resumes its normal, relatively constricted state (inappropriate as compared to an expected dilated state at a T_c comparably elevated passively by heat exposure, but thereby helping to maintain T_c at its febrile level). Since the blood perfusing the skin is now warmer, T_{sk} rises and the skin condition changes to warm and pink; consequently, the earlier sensation of cold disappears. When the fever drive eventually abates, the effector mechanisms evoked resemble those of heat-exposed subjects, viz., T_c falls in conjunction with cutaneous vasodilation and drenching sweating, and the defervescing subject seeks a cooler environment.

It should be apparent from the preceding that the T_c rise of fever is not the unavoidable consequence of the *passive* gain of heat in excess of the capability of *active thermolytic* (heat-dissipating) *effectors* to disperse it; this characterizes ►**hyperthermia**. Fever is, rather, the deliberate result of the *regulated* operation of *active thermogenic* (heat-producing) *effectors*; it thus develops as the result of an upward shift of the thermoregulatory ►**set point**, that is, it represents the regulated adjustment of T_c to a higher than the basal level rather than an uncontrolled side effect of disease; fever is indeed characterized by the active defense of the new, higher T_c . Hyperthermia, thus, is dependent on the T_a , whereas fever can develop at any T_a . A characteristic thermoregulatory behavior that derives from this difference is that febrile subjects prefer warm thermal environments to facilitate heat storage, as already mentioned, whereas hyperthermic subjects choose cool ones to enhance heat loss. Fever is therefore clearly

distinct from hyperthermia, and the two terms should not be used interchangeably.

Various circulatory and respiratory adjustments that serve to support the increased metabolic demands of the heat-producing tissues accompany the febrile rise. These include increases in heart rate, increased blood flow to the thermogenic organs (viz., skeletal muscle, BAT), and associated adjustments and redistributions of the cardiac output. Hyperventilation also occurs transiently, resulting in a fall in $P_{a\text{CO}_2}$ and a rise in pH_a ; $P_{a\text{O}_2}$ is generally unaffected. Other changes involve endocrine, enzymatic, and cellular effectors involved in the provision and utilization of energy; these are analogous to those that sustain the increased heat production on cold exposure.

Pathogenesis of the Febrile Response

Fever arises as the result of a complex, phased sequence of interactions among soluble factors and cells that is initiated in the periphery by the presence of the pathogens or their products and is eventually transmitted to the brain, which modulates the febrile response [2]. The process is driven in the periphery and in the brain via mediators that provide propyretic (e.g., ►**pyrogenic cytokines**, prostaglandin (PG) E_2) and antipyretic (e.g., arginine vasopressin, nitric oxide) signals at different points along the fever pathway and whose sequence is time-dependent. ►**Endogenous antipyresis** is an essential, autoregulatory feedback that serves to prevent an exaggerated fever from occurring during systemic infectious challenges [3]. However, the precise interplay of mediators, their sequence, and their site of action along the route to the brain and/or within it are still incompletely defined.

Many different substances are capable of causing fever; some common pyrogenic stimuli are listed in Table 3. These materials, however, are not the factors that directly induce fever and its nonthermal correlates. Rather, they induce in the host certain immunoregulatory mediators that entrain the APR. Thus, extraneous organisms or their products that have penetrated the body are immediately recognized through their unique molecular patterns (pathogen-associated molecular patterns, PAMPs) by specialized receptors on the host's immune cells. These receptors, called Toll-like receptors (TLRs), occur, predominantly, on mononuclear phagocytes (e.g., circulating and resident macrophages). Their activation transduces the pathogenic microbial signal into intracellular molecular processes that eventuate in the concatenated production of the factors that mediate the APR. LPS acts via the TLR4 receptor; other PAMPs activate other TLRs, but it would appear that the cascades of mediators thus produced are similar.

These propyretic factors belong to the class of immunomodulatory polypeptides called ►**cytokines**. Most prominent among these are interleukins (IL)-1 β

Fever. Table 3 Some common pathogenic stimuli that induce fever ("exogenous pyrogens")

A. Microbial	
•	<i>Viruses</i> (whole organisms; hemagglutinin; dsRNA)
•	<i>Bacteria</i>
•	Gram-positive (whole organisms; peptidoglycans [e.g., muramyl dipeptide]; lipoteichoic acids; exotoxins; enterotoxins; erythrogenic toxins; group B polysaccharides)
•	Gram-negative (whole organisms; peptidoglycans; lipopolysaccharides [lipid A])
•	<i>Mycobacteria</i> (whole organisms; peptidoglycans; polysaccharides; lipoarabinomannan)
•	<i>Fungi</i> (whole yeasts; capsular polysaccharides; proteins)
B. Nonmicrobial	
•	<i>Antigens</i> (e.g., bovine or human serum albumin, bovine gamma globulin, ovalbumin, penicillin)
•	<i>Inflammatory agents</i> (e.g., asbestos, silica, UV radiation, turpentine)
•	<i>Plant lectins</i> (e.g., concanavalin A, phytohemagglutinin)
•	<i>Drugs</i> (e.g., polynucleotides [e.g., polyribonucleoside:polyribocytidylic acid], antitumor agents [e.g., bleomycin], plant alkaloids [e.g., colchicine], synthetic immunoadjuvants [e.g., muramyl peptides])
•	<i>Host-derived</i> (e.g., antigen-antibody complexes, activated complement fragments, inflammatory bile acids, urate crystals, certain androgenic steroid metabolites [e.g., etiocholanolone], certain lymphocyte products)

and -6, tumor necrosis factor (TNF)- α , and interferons (IFN)- α and - γ . Following the administration of LPS, TNF- α normally appears in the bloodstream first, followed by IL-1 β , and finally by IL-6; IFNs do not occur or occur very late – they are induced predominantly in response to viruses and their products. Their functional levels are modulated by the coincident release of their own antagonists, viz., specific target cell-surface antagonists (e.g., IL-1 receptor antagonist), soluble blood-borne receptors (e.g., soluble TNF receptor type II), and/or inhibitors of their synthesis (e.g., glucocorticoids) or their actions (e.g., arginine vasopressin in the POA).

How precisely circulating cytokines trigger the neural circuits that modulate the febrile response is still uncertain. Several possible mechanisms have been proposed: (i) active transport across the blood–brain barrier (BBB), which is otherwise impermeable to proteins; (ii) passage through “leaky portals” in the BBB, the so-called circumventricular organs, especially the *organum vasculosum laminae terminalis* (OVLT) which is located on the midline of the preoptic area (POA) of the anterior hypothalamus, the brain region controlling T_c ; (iii) interaction with endothelial cells in the blood–brain interface, causing the abluminal release of additional cytokines and/or of a further factor, PGE₂, which is considered to be the final, central fever mediator [5]; and (iv) activation of sensory nerves, particularly hepatic vagal and trigeminal afferents. However, because under certain experimental conditions, for example, the i.v. injection of LPS, fever develops before cytokines are detectable in the blood, it has been suggested that, alternatively, circulating LPS could directly trigger endothelial cells in the cerebral microvasculature or in the OVLT, that is, independently of circulating cytokines, via the circulating receptor for LPS, soluble CD14, which can act on these cells

(endothelial cells do not express the membrane-bound LPS receptor), engendering PGE₂. However, again, the synthesis of the enzyme that is thus specifically induced to catalyze the formation of PGE₂ from its substrate, arachidonic acid, cyclooxygenase (COX)-2, significantly lags the onset of fever. Indeed, experimental manipulations that inhibit either the actions of TNF- α or IL-1 β or the production of PGE₂ attenuate the late, but not the early, phases of the febrile response, indicating that cytokines and PGE₂ are more likely involved in the maintenance than in the initiation of fever [2]. Moreover, recent evidence has indicated that the LPS-stimulated secretion of cytokines and PGE₂ by cerebral endothelial cells is polarized luminally, that is, toward the blood, and that the thus activated cells are not localized to the POA region, but nonselectively across the brain. Hence, an alternative, rapid, signaling pathway to the POA must operate in this model. Very recently, evidence was adduced that the fever-triggering factor is PGE₂, very rapidly elaborated not in the POA, but by hepatic macrophages (Kupffer cells, Kc). Two disparate views have been advanced as to what obtains. One holds that the Kc are activated by LPS acting via the TLR4 system, inducing within 30 min COX-2-dependent PGE₂ (COX-2 is constitutively expressed in Kc) which is released into the circulation, coupled to a carrier, albumin, and transported to the brain into which it, as a lipophilic molecule, freely diffuses [5]. There is indeed good evidence that COX-2 is upregulated in the liver and that PGE₂ is detectable in the blood within 30–45 min post-LPS administration, but no compelling evidence exists that blood-borne PGE₂ passes into the POA. The other view argues that the Kc are activated by an immune mediator, complement component C5a, itself activated immediately on contact with LPS, causing the release (within 2 min) of PGE₂ into the liver interstitium; the released PGE₂ then activates

hepatic vagal afferents that project to the POA via noradrenergic connections in the medulla of the brain. Norepinephrine (NE) is consequently very quickly released in the POA, activating two of its receptors, α_1 and α_2 . Stimulation of the first inhibits warm-sensitive neurons in the POA, thereby reducing peripheral heat loss (by inducing cutaneous vasoconstriction) and, hence, causing the first febrile rise and stimulation of the second activates after a ca. 60–90 min delay POA COX-2, inducing the production of PGE₂ and the second phase of fever. Nitric oxide, also concomitantly liberated in the POA, counteracts the release of NE, thereby limiting fever height and duration. All the steps in this sequence have been confirmed by their blockade by cognate antagonists and by vagotomy [8].

Pharmacological Interventions

Fever has been recorded and associated with disease throughout history and, prior to the discovery of infectious pathogens, was taken as an illness in its own right. Now, however, although disagreeable and debilitating, fever is generally considered to be a healing response. Indeed, although no direct link between fever and host survival has definitively been demonstrated, there remains little doubt that it is a defensive response to the invasion of the body by infectious organisms and that its heat is beneficial as an adjuvant to the various immunological functions that are coactivated with it by creating the optimal thermal environment for their precisely timed and patterned expression, particularly during the early phases of the host's responses, that is, the APR [9].

Nevertheless, the use of antipyretics, viz., nonsteroidal anti-inflammatory drugs, such as aspirin, ibuprofen, naproxen, is very popular, particularly since the reduction in T_c is also associated with the relief of the untoward symptoms of sickness behavior, thus moderating the discomfort level and consequently alleviating the anxiety of both the afflicted patients and their caregivers. It is true, however, that the increased metabolic rate associated with fever production could represent a potential strain for those whose energy reserves may be limited, such as the malnourished, elderly, neonates, cancer patients, patients with metabolic diseases, and others. Similarly, the tachycardia and polypnea associated with sustaining the increase in metabolism may put at risk patients with a diminished capacity to increase cardiac work (e.g., congestive heart failure) or to hyperventilate (e.g., chronic obstructive pulmonary disease, asthma). Patients with dysfunctional kidneys may also be imperiled. There is, therefore, merit in attenuating fever in such patients. But, in general, it may be just as well not to treat the T_c rise per se in the absence of high-risk predisposing factors or when the fever is not unduly prolonged or rises to temperatures above 41°C.

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Fibrates

Fibrates are fibric acid derivatives, including e.g. bezafibrate, gemfibrozil, fenofibrate or clofibrate. Fibrates cause a marked reduction in circulating very low density lipoproteins (VLDL) as well as a modest (10%) reduction in low density lipoproteins (LDL) and an approximately 10% increase in high density lipoproteins (HDL). Many of the effects of fibrates on blood lipids are mediated by their interaction with ►[peroxisome proliferator activated receptor \(PPAR\)](#), which regulates gene-transcription in a variety of organs. Fibrates bind to the α -isotype of PPAR (PPAR α), which is expressed primarily in the liver and brown adipose tissue and to a lesser extend in kidney, heart and skeletal muscle. Fibrates reduce triglycerides through PPAR α -mediated stimulation of fatty acid oxidation, increased lipoprotein lipase synthesis and reduced expression of apoC-III. A major side-effect of fibrates is myositis, which is rare but can be severe. Fibrates are clinically used to treat elevated levels of triglycerides.

- [Peroxisome Proliferator-Activated Receptor \(PPARs\)](#)
- [HMG-CoA Reductase Inhibitors](#)

A δ -fibres

A δ -fibres are small diameter myelinated afferent fibres. As part of the pain sensory system they are present in

nerves that innervate the skin and deep somatic and visceral structures.

► Nociception

Fibrin

Fibrin is an elastic filamentous protein elaborated from its precursor, fibrinogen, which is present in plasma at high concentration. Fibrin is formed in response to the actions of thrombin. Thrombin cleaves small peptides from the fibrinogen molecule, forming fibrin monomers that will begin to polymerize and become crosslinked.

► Fibrinolytics

► Coagulation/Thrombosis

Fibrinogen

Fibrinogen, a glycoprotein with an overall homodimeric structure (340 kDa), is synthesized by the liver and secreted into the blood, where its concentration is $\sim 10 \mu\text{M}$. If its N-terminal portion is removed by serine proteases (e.g., thrombin, snake venoms), the remaining part, the fibrin loses its solubility, and forms aggregate (the blood clot, the main structure of a thrombus). The homodimeric structure allows fibrinogen to cross-bridge activated integrin $\alpha\text{IIb}\beta 3$ molecules on adjacent platelets, the crucial step in platelet aggregation.

► Fibrinolytics

► Coagulation/Thrombosis

► Antiplatelet Drugs

Fibrinolytics

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Synonyms

Fibrinolysis; Thrombolysis

Definition

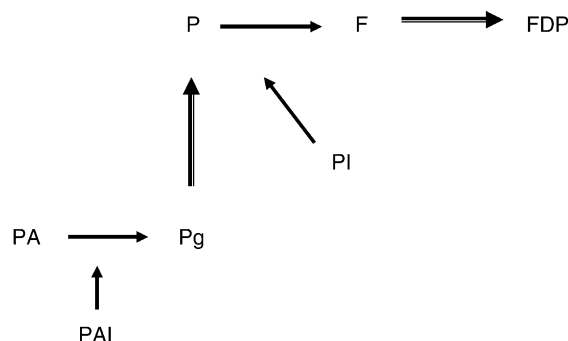
Fibrinolytic enzymes (proteases) are used to dissolve thrombus, the insoluble aggregate of ►fibrin and platelet including several additional cellular and molecular components of the blood.

Mechanism of Action

The term fibrinolysis means the degradation of fibrin (present as an insoluble protein molecule in the blood, which is useful to prevent bleeding and is dangerous if it is formed inside the blood circulation). However, the system in vivo is more complicated because fibrin exists in a compartment with several cellular and molecular components, which modulate fibrinolytic processes. In addition, the fibrinolytic enzymes are synthesized in proenzyme (zymogen) form. Thus, their activation is a prerequisite for their function. Moreover, both the fibrin degradation and the zymogene activation are controlled at multiple levels. Although there are several proteases with the capacity to digest fibrin, such as trypsin, elastase, cathepsin G, extracellular matrix metalloproteinases, in medicine, ►plasmin is considered to be the main fibrinolytic enzyme [1]. A simplified model of the ►plasminogen–plasmin system is illustrated by Fig. 1.

Fibrin Degradation

Fibrin is formed from ►fibrinogen synthesized by the liver and secreted into the circulation. The conversion of fibrinogen to fibrin is initiated by a serine protease, thrombin. Thrombin, at the same time, can activate a transglutaminase enzyme, factor XIII present in



Fibrinolytics. Figure 1 The plasminogen–plasmin system. Plasminogen activators (PA) convert plasminogen (Pg) to plasmin (P), and the latter solubilizes fibrin (F) via digestion, resulting in fibrin degradation products (FDP). Both reactions, the plasmin activity and plasminogen activation, are controlled by inhibitors, such as α_2 -plasmin inhibitor (PI) and plasminogen activator inhibitor-1 (PAI-1), respectively.

the blood bound to fibrinogen. The activated factor XIII (F-XIIIa) thereafter forms crosslinks (isopeptid bonds) between fibrin molecules. In addition, F-XIIIa is able to crosslink α_2 -plasmin inhibitor (α_2 -PI, see later) to fibrin. Thus, at least three forms of fibrin substrate may exist for the fibrinolytic enzyme, plasmin. The rate of their digestion is different; fibrin degradation is more efficient than that of crosslinked fibrin, and the degradation of fibrin crosslinked with α_2 -PI is less efficient [2]. The reactions are illustrated in Fig. 2.

Plasminogen Activation

Plasminogen, a single-chain glycoprotein (92 kDa) is synthesized by hepatocytes and secreted into the blood circulation, where its concentration is fairly stable ($\approx 2 \mu\text{M}$). It is converted to plasmin by plasminogen activators; all plasminogen activators cleave the Arg₅₆₁–Val₅₆₂ peptide bond yielding plasmin (83 kDa), which consists of two chains held together by disulfide bridges. The conformation change in plasminogen during activation results in the appearance of active site (Ser, His, Asp) of the enzyme. Native plasminogen is not a susceptible substrate to activators, however, removal of its N-terminal portion either conformationally (e.g., binding to fibrin) or proteolytically (e.g., hydrolyzing peptide bond at Lys₇₆ by plasmin) makes peptide bond Arg₅₆₁–Val₅₆₂ sensitive to plasminogen activators. The latter reaction, the acceleration of plasminogen conversion to plasmin by plasmin indicates that plasminogen activation is under positive feedback control [2].

Regulation of Plasminogen Activation

There are endogenous and exogenous plasminogen activators. Endothelial cells (and tumor cells) synthesize

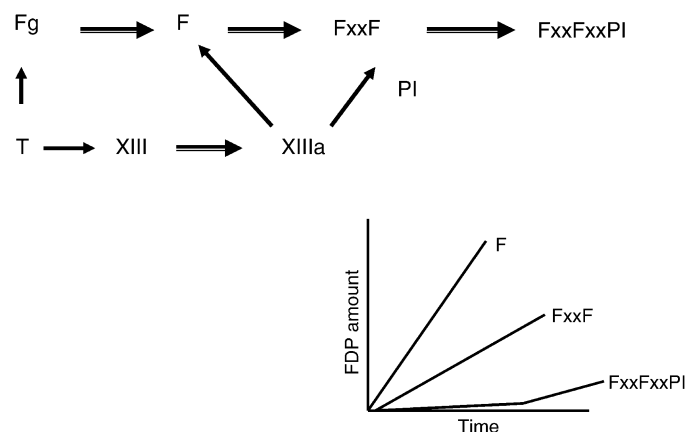
both urokinase-type (uPA) and tissue-type (tPA) plasminogen activators. uPA is produced in a zymogen form, and is converted to active enzyme by plasmin (and kallikrein), this reaction indicates a positive feedback control. tPA is secreted by an active enzyme conformation, which means that it is responsible, very probably, for the initiation of fibrinolysis. tPA is not an efficient enzyme (uPA is more active, approximately by an order of magnitude), however in the presence of cofactors it becomes as efficient as uPA (Fig. 3). Fibrin, the substrate of plasmin, at the same time, is a cofactor for tPA (uPA activity is not affected by fibrin). Besides fibrin, endothelial cell surface, myosin, actin, some extracellular matrix proteins, certain denatured proteins, and additional components may also serve as cofactor for tPA, but their exact *in vivo* role is not clear yet.

The fibrinolytic reactions are controlled by endogenous blood plasma inhibitors as well [3].

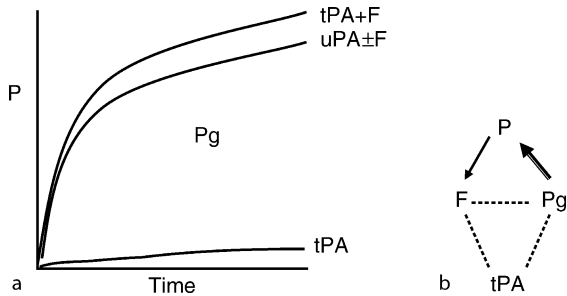
The α_2 -Plasmin Inhibitor

One of the most efficient plasmin inhibitor is α_2 -PI (70 kDa), which is synthesized by the liver, secreted into the blood circulation, where its concentration is $\sim 1 \mu\text{M}$. It rapidly forms equimolar complex with plasmin, and in this complex, the active site of the enzyme is irreversibly blocked. The complex, thereafter, is removed by the liver. It is remarkable that when plasmin is bound to its substrate (fibrin), it is protected against its primary inhibitor, α_2 -PI: the rate of inactivation decreases by ~ 400 -fold (Fig. 4) [3].

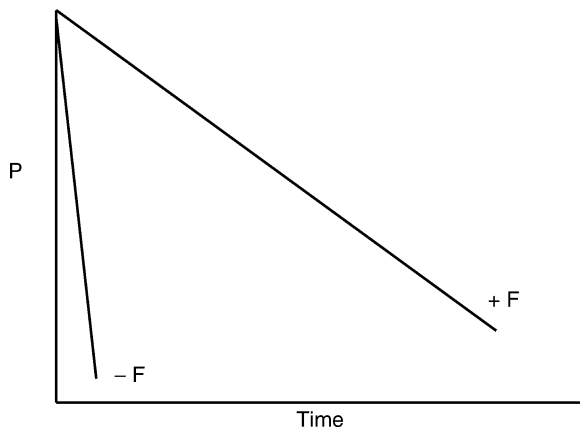
There are several additional plasmin inhibitors in the blood, e.g., α_2 -macroglobulin, α_1 -proteinase inhibitor, antithrombin, but their role in the control of fibrinolysis is questionable, because their action on plasmin is eliminated by fibrin.



Fibrinolytics. Figure 2 Various fibrin structures for plasmin. Fibrinogen (Fg) is converted to fibrin (F) by thrombin (T), and thrombin can also convert factor XIII (XIII) to activated factor XIII (XIIIa). The latter produces crosslinks between fibrins (FxxF) and also may crosslink fibrin with α_2 -plasmin inhibitor (FxxFxxPI). The efficiency of digestion of these plasmin substrates by plasmin, resulting in the soluble fibrin degradation products (FDP), is different. The amount of FDP formed in time is expressed in arbitrary units.



Fibrinolytics. Figure 3 Plasminogen activation (a): Kinetics of plasminogen activation by uPA (urokinase-type) and tPA (tissue-type) plasminogen activators. Effect of fibrin (b): Ternary complex formation between enzyme (tPA), substrate (Pg) and cofactor (F). Abbreviations: plasmin (P), fibrin (F), plasminogen (Pg). Plasmin, formed in time, is expressed in arbitrary units.



Fibrinolytics. Figure 4 Inactivation of plasmin by α_2 -plasmin inhibitor: Effect of fibrin. The inactivation rate of free plasmin is very rapid (the second order rate constant: $k'' \approx 430 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$), while of fibrin bound plasmin is slow (the second order rate constant: $k'' \approx 1 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$). Inactivation of plasmin in the figure is shown in arbitrary units. Abbreviations: plasmin (P), fibrin (F).

Termination of Plasminogen Activation

There are several blood plasma inhibitors for plasminogen activators as well. Among them, the most significant is the plasminogen activator inhibitor-1 (PAI-1). It is a glycoprotein (52 kDa), synthesized by endothelial cells and is present in the blood circulation (mainly in platelets) at varying concentration up to 2 nM. This inhibitor forms equimolar complexes with both tPA and uPA, where the enzymes lose their activity. The regulation of the amount of plasminogen activators and PAI-1 is not clearly known.

A Compartmental Approach to Fibrinolysis

Similarly to blood coagulation, reactions of fibrinolysis occur on the interface of fluid-and solid-phase structures, generally in transiently formed compartments.

Enzymology of proteases in a water-phase is well known, but its alteration in a compartment is poorly understood. There are dramatical changes in reaction rates, in enzyme contractions and in enzyme sensitivity to inhibitors, which are not exactly described. In addition, besides fibrin and platelets there are several cellular and molecular components present in a thrombus compartment, where their influence on the basic fibrinolytic reactions is not known. To study this aspect of fibrinolysis is a task of the near future [4].

Clinical Use

Since plasmin in free form (not bound to fibrin) is extremely and rapidly inactivated by the inhibitor system (Fig. 4), plasminogen activators are used for treatment of thrombosis. Under such a condition, if plasmin is formed by the activators (especially by tPA) at the site of fibrin, the bound form can degrade fibrin because it is “protected” against the inhibitor system. In the medical practice, mainly two endogenous plasminogen activators, tPA and uPA, and one exogenous, the **streptokinase (SK)** are used [1,4].

Tissue-Type Plasminogen Activator

The **tissue-type plasminogen activator (tPA)** is a single-chain glycoprotein (sctPA) with 68 kDa molecular mass, present in blood at ~ 60 pM concentration (20% in free form, 80% in a complex with PAI-1). The N-terminal portion of tPA consists of a finger, an epidermal growth factor (EGF) and two kringle domains. Its C-terminal part comprises the catalytic domain, which is homogenous to that of other trypsin-like serine proteases. The sctPA can be converted by plasmin to a two-chain form (tctPA), but the biological significance of this modification is not known. tPA levels in vivo are influenced by various factors, such as hormones, exercise etc., but the exact control of its synthesis and release is not well known. tPA is also produced by engineering technique and used as fibrinolytic drug. During applications, the PAI-1 level should be overtitrated by tPA, which is not a problem, because PAI-1 concentration is low.

Urokinase-Type Plasminogen Activator

The **urokinase-type plasminogen activator (UPA, urokinase)** is a single-chain glycoprotein with 55 kDa molecular mass, produced by variety of cells, among them endothelial and tumor cells, and is present at ~ 70 pM in blood plasma. It consists of an EGF domain (responsible for binding to cell surface receptors), a kringle and a catalytic domain (the latter homologous to the trypsin-like proteases). Single-chain uPA (scuPA) has no catalytic activity, but following cleavage at the $\text{Lys}_{158}\text{--Ile}_{159}$ bond by plasmin (or kallikrein), the emerged two-chain form held together by S–S bridges (tcuPA) is a potent plasminogen activator. Thus, uPA

can contribute mainly to amplification of fibrinolysis. The main problem in the therapeutical application of uPA is that it activates plasminogen randomly (see (SK) as well).

Streptokinase

SK is a single-chain protein (47 kDa), produced by *Streptococcus hemolyticus*. SK is not an enzyme, but forms a complex with human plasminogen, and in the complex an active site of plasminogen develops, which acts as a plasminogen activator, converting a neighboring free plasminogen to plasmin. There are two problems with SK treatments. It activates plasminogen (like uPA) efficiently but randomly, thus fibrinolytic therapy is generally efficient only, when ► α_2 -plasmin inhibitor is consumed (blood concentration of plasminogen and its inhibitor is 2 μ M and 1 μ M, respectively), which on the other hand, may cause bleeding complication. In the future, perhaps a fibrin-dependent form of SK will be developed. If the N-terminal residues (59 amino acids) of SK are removed, the remaining portion becomes fibrin-dependent plasminogen activator in a complex with plasminogen. An additional problem with SK is that as a foreign protein it may provoke immunological complications, anaphylactic reactions, when it is used again.

There are several other sources and recombinant variations of plasminogen activators as well. To illustrate them, only a few examples are mentioned. Blood coagulation factor XIIa can activate plasminogen, although not efficiently, but perhaps it may play a role in the initiation of fibrinolysis. It needs additional works. Staphylokinase (15 kDa), synthesized by *Staphylococcus aureus*, somehow is similar to SK, it also interacts with human plasminogen. Recombinant derivatives of tPA and uPA also exist. For example, reteplase, a tPA variant, consists of the kringle-2 and protease domains (lack of the finger and the kringle-1 domains) of the wild-type activator. Their mechanism of action and especially their possible clinical application are under investigation.

Summarizing the fibrinolytic therapy, it should be emphasized that efficient treatment needs urgent application of plasminogen activator (within a few hours) to prevent the formation of crosslinks in the fibrin structure (Fig. 2) and to find the localization of thrombus to emerge plasmin on the surface of fibrin to prevent rapid inactivation of the enzyme by the inhibitor system of fibrinolysis (Fig. 3).

- Anticoagulants
- Coagulation/Thrombosis

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Fibroblast Growth Factors

Fibroblast growth factors (FGFs) are a group of about 20 growth factors, which function through a group of receptor tyrosine kinases (FGF-R-1, -2, -3 and -4). They play multiple roles in the morphogenesis and growth of higher organisms.

- Growth Factors

Filamin

Also called filamin human actin-binding protein (ABP). A 280 kd dimeric actin-crosslinking protein that plays a key role in the anchoring of membrane proteins to the actin cytoskeleton and is responsible for the crosslinking of actin filaments into orthogonal networks in the cytoplasm. It is comprised of three functional domains: an N-terminal filamentous actin-binding domain, a C-terminal self-association domain, and a membrane glycoprotein-binding domain. As a protein involved in the remodeling of the cytoskeleton, Filamin A is central to the modulation of cell shape and migration.

- Cytoskeleton
- Transforming Growth Factor-Beta

First-order Kinetics

First order kinetics describes the most common time course of drug elimination. The amount eliminated within a time-interval is proportionate to the drug concentration in the blood.

- Pharmacokinetics

First-pass (Presystemic) Metabolism

First-pass metabolism is the elimination of an orally administered drug by the liver or sometimes the gut wall, before it reaches the systemic circulation. First-pass metabolism results in a decreased systemic bioavailability.

► Pharmacokinetics

FK506

Synonym

Tacrolimus

Definition

A secondary metabolite produced by *Streptomyces tsukubaensis*. This bacterium was initially isolated in a soil sample collected in Japan. FK506 is a macrocyclic lactone and shares structural similarity with rapamycin. Consistently, both rapamycin and FK506 bind the same immunophilin FKBP12 (FK506 binding protein of 12 kDa). However, unlike rapamycin-FKBP12 which binds and inhibits the TOR kinase, FK506-FKBP12 binds and inhibits the protein phosphatase calcineurin.

FK506 is clinically used as immunosuppressant.

- TOR Signalling
- Immunosuppressive Agents
- Protein Phosphatases

FKBP12

Synonyms

12 kDa FK506-binding protein

Definition

FKBP12 is a member of immunophilin family that has prolyl isomerase activity and is related to the cyclophilins in function. FKBP12 binds immunosuppressant molecule FK506 (tacrolimus). The FKBP-FK506 complex inhibits calcineurin, a protein phosphatase, thus blocking signal transduction in the T-lymphocyte

transduction pathway. In addition, FKBP12 binds to ryanodine receptor/ Ca^{2+} -induced Ca^{2+} release channel to modulate its function.

- FK506
- Immunosuppressive Agents
- Protein Phosphatases
- Ryanodine Receptor

Flare

The surrounding redness caused by the vasodilatation of local blood vessels in the skin (hyperaemia). Histamine released at the site of contact acts on sensory nerve endings in the skin. Impluses travel along the axon to other peripheral branches of the same neuron to cause release of vasodilatory peptide neurotransmitters from nerve endings serving a wider area of skin than the initial contact point. Impluses reaching the CNS are interpreted as itch and pain.

► Histaminergic System

Flavin Adenine Dinucleotide (FAD)

Flavin Adenine Dinucleotide (FAD) ($\text{C}_{27} \text{H}_{33} \text{N}_9 \text{O}_{15} \text{P}_2$) is a coenzyme that acts as a hydrogen acceptor in dehydrogenation reactions in an oxidized or reduced form. FAD is one of the primary cofactors in biological redox reactions.

- Vitamins B2
- Monoamine Oxidases and their Inhibitors

Flavin Mononucleotide (FMN)

Flavin Mononucleotide (FMN) ($\text{C}_{17} \text{H}_{21} \text{N}_4 \text{O}_9 \text{P}$) is a phosphoric ester of riboflavin that constitutes the cofactor of various flavoproteins.

► Vitamins B2

Flp/FRT

Flp/FRT is a system analogous to the cre/loxP system. Flp is a yeast enzyme that recognizes FRT sites. If two FRT sites have a parallel orientation, the DNA segment between these sites will be deleted by the action of the Flp recombinase.

► Transgenic Animal Models

Fluorescence in situ Hybridization (FISH)

A diagnostic method using fluorescence labeled DNA probes to detect and quantify the number complementary chromosomal sequences on a cellular resolution. A related technique that also allows assessment of gene amplifications, but without precise quantification of copy numbers is the chromogenic in situ hybridization (CISH). Here, instead of a fluorescent dye an enzyme that can generate a colored precipitate in the tissue samples is coupled to the DNA probe.

Fluoride

Fluoride forms a tetrahedral ion with aluminium, AlF_4^{B} , which forms a complex with the $\text{GDP}\alpha\beta\gamma$ form of G-proteins. In the case of G_s , the complex $\text{AlF}_4^{\text{B}}\text{CGDP}$ behaves much as GTP or the more stable GTP derivatives, $\text{GTP}\gamma\text{s}$ or $\text{GPP}(\text{NH})\text{p}$, and causes activation of adenylyl cyclase through the complex $\text{AlF}_4^{\text{B}}\text{-XGDP}\alpha_s\text{XC}$.

Fluoride stimulates bone formation by protein kinase activation mediated effects on osteoblasts. Fluorides have been used in the treatment of osteoporosis, but their anti-fracture effect is not undisputed.

- Heterotrimeric G-Proteins
- Adenylyl Cyclases
- Bone Metabolism

Fluoroquinolones

A fluorine atom in position 6 of the basic structure of quinolones enhances the antimicrobial activity considerably. All widely used quinolones are fluorinated in position 6 and the term “fluoroquinolones” is often used to describe these drugs. However, some new quinolones with similar antimicrobial activity are not fluorinated in position 6 (e.g. garenoxacin, PGE9262932) and therefore the term “quinolones” is more appropriate to describe this group of antimicrobial agents.

► Quinolones

FMN

- Flavin Mononucleotide
- Vitamin B2

Foam Cells

Cells in the atheroma derived from both macrophages and smooth muscle cells that have accumulated modified low-density lipoproteins. Their cytoplasm laden with lipid causes the “foamy” appearance on microscopy

► Atherosclerosis

Folate

- Folic Acid
- Dihydrofolate Reductase

Folic Acid

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Synonyms

Folate

Definition

Folic acid is sensitive to photodegradation as well as oxidative degradation and as an acid only slightly soluble in water, but in the salt form well soluble. 5,6,7,8-Tetrahydro-folic acid (H₄PteGlu) is derived from folic acid by two consecutive reductions using NADPH₂. H₄PteGlu and its derivatives are the biologically active vitamers. Reduced folates (H₂PteGlu or H₄PteGlu) are less stable than folic acid itself. Folate losses during food preparation are high, especially when foods are excessively heated or soaked in water. Bioavailability of natural folate from foods averages 50%, while folic acid from supplements or fortified foods is absorbed to more than 90%.

Spinach, salad, cereal germ, and bran as well as pulses are good sources of folic acid. Liver and yeast contain high amounts of this vitamin, too, but are not consumed frequently enough to be relevant for the coverage of daily requirements [1,2].

Folic acid or folate (Fig. 1) is the collective name for more than 100 derivatives of pteroyl-mono-L-glutamate. In plant and animal tissues, folic acid mostly occurs as pteroyl-oligo-L-glutamate (PteGlu_n), with up to eight glutamyl residues.

Mechanism of Action

Tetrahydrofolic acid (H₄PteGlu) accepts and transfers activated one-carbon units in the form of 5-methyl-,

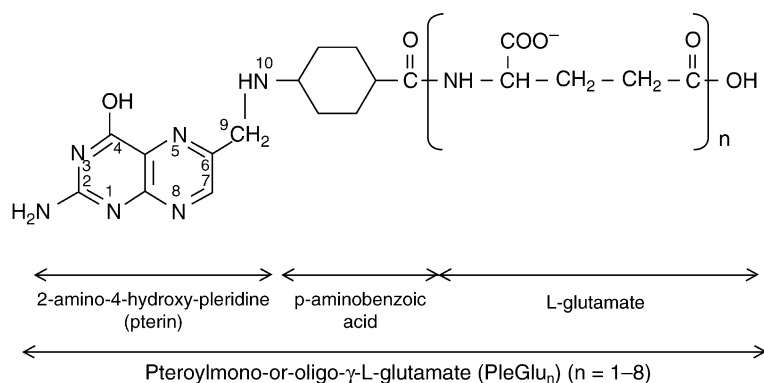
10-formyl-, 5-formyl-, 5,10-methenyl-, 5,10-methylene-, and 5-formiminotetrahydrofolate (Fig. 2). These activated metabolites are involved in the methylation of homocysteine to methionine, the conversion of glycine to serine, in histidine metabolism as well as in choline, purine, and pyrimidine biosynthesis.

5-Methyl-tetrahydro folic acid is furthermore, together with vitamin B12 and B6, required to regenerate homocysteine (see ►Vitamin B12, Fig. 1). Homocysteine results when methionine is used as a substrate for methyl group transfer. During the last few years, homocysteine has been acknowledged as an independent risk factor in ►atherosclerosis etiology. Folic acid supplementation can help reduce elevated homocysteine plasma levels and is therefore supposed to reduce the risk of atherosclerosis as well [2].

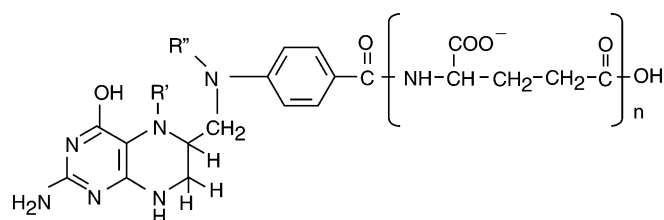
Clinical Use (Including Side Effects)

Folic acid deficiency is common even in industrial countries. High-risk groups to develop folic acid deficiency are pregnant and breastfeeding women, people who regularly take anticonvulsant drugs, oral ►contraceptives, or tuberculostatics, patients suffering from malabsorption, and chronic alcoholics. As folic acid is involved in cell proliferation, deficiency symptoms first become evident in tissues and cells with high proliferation rates, such as erythrocytes and epithelia.

It is recommended that women of childbearing age take 400 µg/d synthetic folic acid as a supplement in order to reduce the risk of neural tube defects of the embryo when they later become pregnant (periconceptional folic acid supplementation) [2]. When supplementing folic acid, it should be considered that this vitamin can mask the simultaneous presence of vitamin B12 deficiency. The typical symptom of vitamin B12 deficiency, megaloblastic (= macrocytic) anemia, will be reduced by high doses of folic acid, yet the nervous system will – in the long run – be irreversibly damaged (= ►funicular myelitis) when vitamin B12 is not provided as well.



Folic Acid. Figure 1 Structure of folic acid.



Coenzyme	R'	R''
Tetrahydro folic acid	-H	-H
5-methyl-tetrahydro folic acid	-CH ₃	-H
5,10-methylene-tetrahydro folic acid		-CH ₂ -
5,10-methenyl-tetrahydro folic acid		-CH=
5-formyl-tetrahydro folic acid	-HCO	-H
10-formyl-tetrahydro folic acid	-H	-HCO
5-formimino-tetrahydro folic acid	-HCNH	-H

Folic Acid. Figure 2 Coenzyme forms of folic acid.

Overall, supplementation with folic acid is considered safe as the vitamin has low acute and chronic toxicity.

- Biotin
- Niacin
- Pantothenic Acid
- Vitamin B1
- Vitamin B2
- Vitamin B6
- Vitamin B12
- Vitamin C
- Dihydrofolate Reductase
- Antiplatelet Drugs
- Antineoplastic Agents

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Follicle-stimulating Hormone (FSH, Folitrophin)

- Contraceptives

Force Fields/Molecular Mechanics

Force field methods, also called molecular mechanics, are empirical approaches to calculate molecular geometries and energies. The general aim of a force field

calculation is to find that conformation of the 3D-dimensional structure of a molecule or complex with the minimal energy. The acting forces between the atoms are described by analytical functions with customisable parameters. Covalent as well as noncovalent forces are considered.

The basic idea of force fields is the assumption that bond length and bond angles adjusts whenever possible to standard values. Steric hindrance of nonbonded atoms can cause nonideal values of bond length and angle. The repulsive interaction is called van der Waals interaction. A force field equation to calculate the energy of the structure for a molecule contains at least the terms van der Waals interaction, bond length stretching, angle deformation and torsion angle deformations. Many force fields contain additional terms like electrostatic attraction and others. The derived force field for each term is achieved by calibration on experimental structural data, quantum-chemical calculation and, if included, charge-type calculations. There are a variety of different force fields calibrated for certain type of molecules and solutes. Among the force fields for proteins the AMBER force field is suitable for protein calculations in vacuum and water, the GRO-MACS force field is suitable for proteins in water and lipid environments.

- Molecular Modeling
- Bioinformatics

Forskolin

Forskolin is a diterpene derivative from the plant *Coleus forskohlii*. It activates all mammalian isozymes of adenylyl cyclase except AC9 and AC10.

Active derivatives of forskolin include: 7-deacetyl-forskolin ($EC_{50} \sim 20\mu M$), 6-acetyl-7-deacetyl-forskolin ($EC_{50} \sim 40\mu M$), 7-deacetyl-7-O-hemisuccinyl-forskolin ($EC_{50} \sim 50\mu M$). The last of these has been used as an immobilized affinity chromatography ligand for the purification of adenylyl cyclases from tissues.

► Adenylyl Cyclases

FoxO1a

FoxO1a (previously known as “FKHR”, synonym “forkhead in rhabdomyosarcoma”, gene name: FOXO1a) is a transcription factor which is regulated by phosphorylation of three serine/threonine residues. Phosphorylation of these residues by the protein kinase Akt leads to inactivation and nuclear exclusion of FoxO1a. Insulin regulates FoxO1a-dependent transactivation of gene expression through activation of Akt. FoxO1a is thought to bind to a conserved consensus sequence (T(G/A)TTT) found in the promoter region of several insulin responsive genes. The promoter of the glucose-6-phosphatase contains three of these FoxO1a binding sites.

► Insulin Receptor

Frizzled

Receptor for wingless ligands.

► Wnt Signalling
► Low-Density Lipoprotein Receptor Gene Family

Frontal Cortex

The frontal cortex consists of three main structures: (i) motoric regions (including the Broca area and ocular areas), (ii) the prefrontal cortex, and (iii) the orbital cortex. The prefrontal cortex is associated with attention, arousal, and expectation, the orbital cortex with motivation.

► Psychostimulants

FSH

Follicle-stimulating Hormone.

► Contraceptives

Functional Genomics

Functional genomics (sometimes referred to as functional proteomics) aims at determining the function of the proteome (the protein complement encoded by an organism's entire genome). It expands the scope of biological investigation from studying single genes or proteins to studying all genes or proteins at once in a systematic fashion, using large-scale experimental methodologies combined with statistical analysis of the results.

► Bioinformatics
► Gene Expression Analysis
► Microarray Technology
► Pharmacogenetics
► Proteomics

Functional Magnetic Resonance Imaging (fMRI)

Brain imaging technique that allows visualization of the brain, in order to understand which brain regions are involved in specific functions. Its functioning is based on the measurement of the regional cerebral blood flow which increases when a specific brain region is activated. Its use is similar to that of positron emission tomography (PET).

► Placebo Effect

Fungi

Fungi (Mycophyta, Mycota, Eumycetes) are chlorophyll-free plants, eukaryotic cells growing in hyphae or yeasts and causing diseases in plants, animals and humans.

► Antifungal Drugs

Fungicidal Effect

A fungicidal effect is that which kills the fungal cell.

► Antifungal Drugs

Fungistasis

Fungistasis is the inhibition of fungal growth without killing the fungal cell.

► Antifungal Drugs

Funicular Myelitis

The neurological disorder associated with severe vitamin B12 deficiency is termed funicular myelitis. Vitamin B12 deficiency leads to disturbed choline-, phospholipid-, and nucleic-acid synthesis, resulting in spinal marrow damages. Disturbed myelin synthesis finally causes irreversible neurological failure. In addition, there are psychiatric disturbances (disturbed memory, apathy).

► Vitamin B12 ► Folic Acid

Furin

Furin, also known as paired basic amino-acid-cleaving enzyme (PACE), is a membrane bound subtilisin-like serine protease of the *trans* Golgi compartment. It is ubiquitously expressed and mediates processing of many protein precursors at Arg-X-Lys/Arg-Arg sites.

So far, seven mammalian precursor convertases (PCs) have been identified: furin, PC1, PC2, PC4, PC5, PACE4 and PC7.

► Somatostatin

Furin-like Protease

A furin-like protease is a precursor (preprohormone, preprotein) convertase (PC).

► Endothelins ► Furin

Fyn

Fyn is a nonreceptor tyrosine kinase related to Src that is frequently found in cell junctions. The protein is N-myristoylated and palmitoylated and thereby becomes associated with caveolae-like membrane microdomains. Fyn can interact with a variety of other signaling molecules and control a diversity of biological processes such as T cell receptor signaling, regulation of brain function, and adhesion mediated signaling.

► Tyrosine Kinases ► Cadherins/Catenins

FYVE Domain

The FYVE domain is a phosphatidylinositol-3-phosphate-binding module of approximately 60 to 80 amino acids. It was named after the first four proteins, where this domain was described (Fab1p, YOTB, Vac1p and EEA1).

► Phospholipid Kinase

Fz Receptors

Frizzled (Fz) proteins comprise a family of seven-pass transmembrane receptors with a cysteine-rich extracellular domain. As a class, Fz proteins are structurally related to the superfamily of heterotrimeric **► G-protein coupled receptors** (GPCRs). There are 4 *Fz* genes in *Drosophila* and 10 in humans, with close orthologs

in mice. Fz proteins participate in both Wnt/ β -catenin-dependent and Wnt/non- β -catenin signaling, but individual Fz receptors may differ in their basal (minus ligand) ability to activate signaling of each type, suggesting that structural differences among the Fz proteins contribute to functional specificity. There is contradictory evidence regarding the importance of the Fz extracellular domain for this specificity, but it is clear that the carboxyl-terminal tail and intracellular loops

contribute. There is evidence that other members of the seven-pass transmembrane receptor superfamily act as multimers on the cell surface. In the case of the Fz receptors there is no direct functional evidence for this, but crystallization studies have revealed a conserved dimerization interface in the extracellular cysteine-rich domain.

► [Wnt Signaling](#)

G-actin

G-actin (globular actin) has a molecular weight of about 42 kDa. In higher vertebrates, six isoforms of G-actin, which contain 374/375 residues, are expressed in a cell-specific manner. They are present in striated muscle cells (skeletal and cardiac isoforms), smooth muscle cells (vascular and visceral isoforms) and in non-muscle cells (two isoforms).

► Cytoskeleton

G-Proteins

► Heterotrimeric G-proteins
► Table Appendix: Receptor Proteins

GABA

γ -Aminobutyric acid.

► GABAergic System

GABA_A Receptors

GABA_A receptors are pentameric complexes on the postsynaptic membrane with a central pore with selectivity for chloride ions. Benzodiazepines and barbiturates increase the GABA-induced chloride currents, leading to hyperpolarization of the postsynaptic membrane.

► GABAergic System
► Benzodiazepines
► Sleep
► General Anaesthetics
► Glycine Receptors
► Table appendix: Receptor Proteins

GABA_B Receptors

Definition

GABA_B receptors mediate the slow and prolonged physiological effects of the inhibitory neurotransmitter GABA. Functional GABA_B receptors are comprised of two subunits, GABA_BR1 and GABA_BR2. Both subunits are ► [G-protein-coupled receptors](#), which couple to the Gi/o family and are densely expressed at spinal nociceptive synapses.

► GABAergic System
► Pain and Nociception
► Analgesics

GABAergic System

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Definition

GABA (γ -aminobutyric acid) is an amino acid with mostly inhibitory functions in the mammalian central nervous system. Structures involved in releasing or binding GABA as a neurotransmitter constitute the GABAergic system. The GABAergic system is involved

in the regulation of vigilance, anxiety, muscle tension, epileptogenic activity and memory functions.

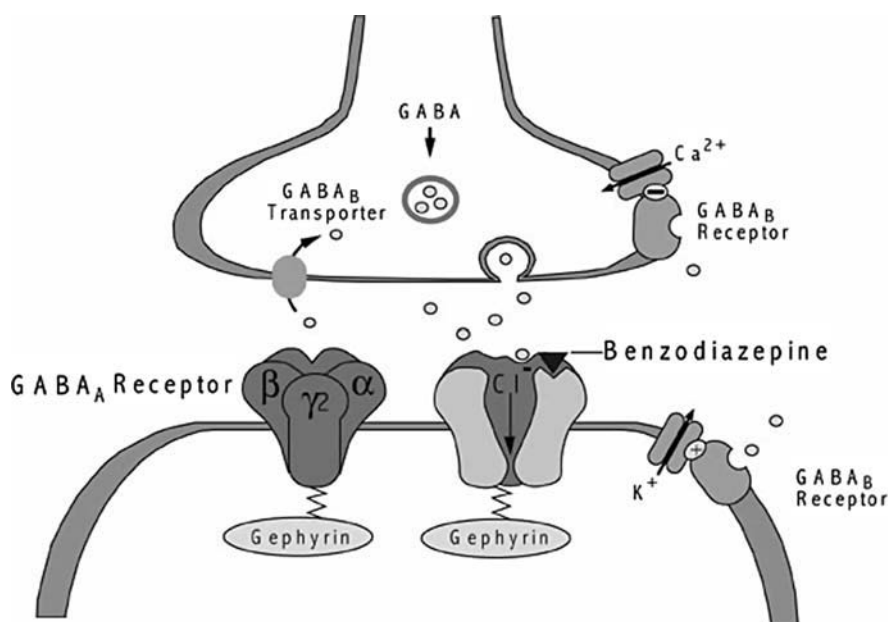
Basic Characteristics

GABA is the major inhibitory neurotransmitter in the mammalian central nervous system. It is synthesized in presynaptic terminals from glutamate by the action of the enzyme glutamic acid decarboxylase, stored in vesicles and released upon the arrival of an action potential. GABA binds to and mediates its effects via postsynaptic ionotropic GABA_A receptors and pre- and postsynaptic metabotropic GABA_B receptors (Fig. 1). Whereas the GABA_A receptors mediate fast responses, the GABA_B receptors mediate slow responses. GABA is removed from the synaptic cleft by GABA transporters and metabolized in a transamination reaction.

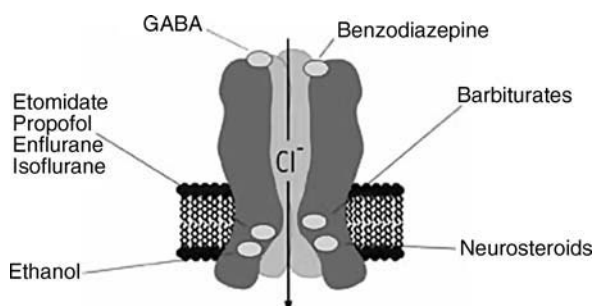
GABA_A Receptors

GABA_A receptors are pentameric membrane protein complexes that operate as GABA-gated chloride channels. They belong to the superfamily of ligand-gated ion channels. They have an extracellular N-terminal domain, four putative transmembrane domains and an extracellular C-terminal domain. The third intracellular loop contains consensus sequences for phosphorylation by protein kinases. The second transmembrane domain presumably lines the channel. On the cytoplasmic side, most GABA_A receptors are indirectly linked to the cytoskeletal protein gephyrin via the $\gamma 2$ subunit; these two components have been shown to play a role in

synaptic clustering of defined GABA_A receptors. The GABA_A receptor subunits are drawn from seven classes with mostly multiple variants ($\alpha 1$ – $\alpha 6$, $\gamma 1$ – $\gamma 3$, $\rho 1$ – $\rho 3$, δ , ϵ , θ). The α subunits share greater than 70% amino acid sequence identity, whereas from one subunit class to the other, e.g. α and γ , the amino acid sequence identity is in the range of ca. 30–40%. Most GABA_A receptors are composed of α , β and γ subunits with $\alpha 1\beta 2, \gamma 2$ being the most abundant receptor subtype. Some evidence suggests that the pentamers may contain two α subunits, two β subunits and one γ subunit. GABA_A receptors are responsible for the fast synaptic inhibition. Binding of GABA to the receptor is followed in a matter of milliseconds by a chloride influx, leading – in most cases – to hyperpolarization and thus functional inhibition of the postsynaptic neuron. GABA_A receptors are of physiological relevance because they play an essential role in the regulation of the excitability of the brain. They are, in addition, of pharmacological relevance since their activity is modulated by a variety of therapeutic agents. These include benzodiazepines, barbiturates, neurosteroids and general anaesthetics (Fig. 2). A subset of GABA_A receptors is frequently referred to as “GABA_C” receptor [2]. These receptors are composed of ρ subunits, which only assemble with each other and are found primarily in the retina. In contrast to the typical GABA_A receptors, these receptors are insensitive to the classical GABA_A receptor antagonist bicuculline. In the IUPHAR nomenclature [3] these receptors are classified as GABA_A receptors.



GABAergic System. Figure 1 GABAergic synapse. Schematic of a GABAergic synapse, depicting the major elements of signal transduction. Postsynaptic GABA_A receptors are pentameric ligand-gated ion channels assembled from various types of subunits. On the cytoplasmic site they are indirectly linked to gephyrin. GABA_B receptor occur pre- and postsynaptically and are coupled to calcium or potassium channels.



GABAergic System. Figure 2 Model of a GABA_A receptor and its binding sites. In addition to the binding site for the neurotransmitter GABA, GABA_A receptors have modulatory binding sites for a variety of ligands including benzodiazepines, barbiturates, neurosteroids, ethanol and general anaesthetics such as isoflurane, enflurane, etomidate and propofol. The positioning and size of the binding sites is arbitrary. One subunit has been removed to visualize the pore.

GABA_A receptors are widely expressed in the central nervous system [4], balancing the excitatory neurotransmission. The GABA_A receptor subtypes display a differential distribution. The $\alpha 1$ subunit, which is by far the most abundant α subunit, is expressed e.g. in cerebral cortex, hippocampus and thalamus, the $\alpha 2$ subunit e.g. in hippocampus, striatum and amygdala, the $\alpha 3$ subunit e.g. in monoaminergic and serotonergic neurons of the brain stem, in basal forebrain cholinergic neurons and in the reticular nucleus of the thalamus, the $\alpha 4$ subunit e.g. in the thalamus, the $\alpha 5$ subunit e.g. in the hippocampus, and the $\alpha 6$ subunit in cerebellar granule cells. Among the β subunits, the $\beta 2$ subunit is the most abundant, followed by the $\beta 3$ and $\beta 1$ subunits. The by far most abundant γ subunit is $\gamma 2$, whereas $\gamma 1$ and $\gamma 3$ are rare. In some instances, receptors contain a δ subunit presumably instead of a γ subunit. The GABA binding site is most likely located at the interface between α and β subunits. The binding site for modulatory benzodiazepines is, however, most likely located at the interface between α and γ subunits. Recombinant receptors consisting of α and γ subunits are only activated by GABA, but not modulated by benzodiazepines. The GABA_A receptors containing the $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ subunits (in addition to β and γ subunits) are sensitive to modulation by classical benzodiazepines such as diazepam, whereas receptors containing the $\alpha 4$ and $\alpha 6$ subunits are not. The latter receptors are also referred to as “diazepam-insensitive” GABA_A receptors. Whereas the diazepam-sensitive α subunits $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ contain a histidine residue at a conserved position in the N-terminal extracellular region (positions $\alpha 1$ -H101, $\alpha 2$ -H101, $\alpha 3$ -H126 and $\alpha 5$ -H105), the diazepam-insensitive $\alpha 4$ and $\alpha 6$ subunits contain an arginine residue in the corresponding position ($\alpha 4$ -R99 and $\alpha 6$ -R100). Mutational analysis revealed that the

presence of a histidine residue or an arginine residue at this position in the α subunit determines whether the respective GABA receptor is diazepam-sensitive or diazepam-insensitive, respectively. Depending on their sensitivity to CL218872, GABA_A receptors can be further classified into those having a high affinity for CL218872 ($\alpha 1\beta \times \gamma 2$) and those having a low affinity for CL218872 ($\alpha 2\beta \times \gamma 2$, $\alpha 3\beta \times \gamma 2$ or $\alpha 5\beta \times \gamma 2$). A glycine in position 201 of the $\alpha 1$ subunit has been found to be necessary for high affinity binding of CL218872 to the respective receptor. The imidazopyridine zolpidem, which binds to the benzodiazepine site, has intermediate affinity for different GABA_A receptor subtypes: It has a high affinity for $\alpha 1\beta \times \gamma 2$, $\alpha 2\beta \times \gamma 2$ and $\alpha 3\beta \times \gamma 2$ receptors, but essentially no affinity for $\alpha 5\beta \times \gamma 2$ receptors. The rare $\gamma 3$ subunit may also confer zolpidem-insensitivity to GABA_A receptors.

Pharmacological Intervention

Several groups of CNS active drugs exert all or some of their clinical effects by their action on the GABAergic system.

Benzodiazepines

► **Benzodiazepines** act by shifting the GABA dose-response curve to the left and thus increase the affinity of the receptors for GABA. At a given concentration of GABA in a synapse, the chloride current will be increased. Benzodiazepines have no action in the absence of GABA (use-dependence) and cannot increase maximal physiological stimulation by a high concentration of GABA, i.e. their action is self-limiting, which most likely contributes to the safety of these drugs with respect to overdoses. They are used as anxiolytics, sedatives, hypnotics, anticonvulsants and central muscle relaxants. At the molecular level, benzodiazepines increase the opening frequency and thus the number of channels that are opened by a given concentration of GABA.

By introducing histidine to arginine point mutations in the $\alpha 1$, $\alpha 2$ and $\alpha 3$ subunits the respective GABA_A receptors are rendered diazepam-insensitive. Thus, it was found that the sedative, the anterograde amnesic and in part the anticonvulsant actions of diazepam are mediated by GABA_A receptors containing the $\alpha 1$ subunit, whereas its anxiolytic and muscle relaxant actions are mediated by GABA_A receptors containing the $\alpha 2$ subunit [5]. The anxiolytic action is observed at much lower concentrations than the muscle relaxant action.

Barbiturates

The binding site for ► **barbiturates** on the GABA_A receptor is less well defined. Barbiturates act by increasing the conductance level. In contrast to benzodiazepines, they also display direct agonistic action on GABA_A receptors. Also in contrast to

benzodiazepines, their action is not self-limiting, i.e. they can activate the GABA_A receptor to higher levels than high concentrations of GABA alone. These features may be responsible for the fact that overdoses of barbiturates are life-threatening.

General Anaesthetics

Both volatile and intravenous anaesthetics have been shown to modulate the activity of the GABA_A receptor, and the assumption is reasonable that these actions may contribute to at least some of the clinical effects of general anaesthetics. Though a binding site for general anaesthetics on the GABA_A receptor is still elusive, several mutations in the second and third transmembrane regions of α and β subunits have been identified that can abolish or inhibit the action of general anaesthetics. These include the volatile anaesthetics isoflurane and enflurane and the intravenous anaesthetics propofol, etomidate and also barbiturates. Interestingly, these mutations may affect the agonistic and the modulatory actions of general anaesthetics independently. Whereas mutations in both α and β subunits inhibit the actions of isoflurane and enflurane on the GABA_A receptor, only mutations in the β subunits inhibit the actions of propofol and etomidate. Since many general anaesthetics, in particular, the volatile anaesthetics also act on other excitatory and inhibitory neurotransmitters and also on two-pore domain potassium channels (background channels), the contribution of the GABA_A receptor system as a whole and specific GABA_A receptor subtypes in particular to certain anaesthetic endpoints is not known and may be different for each drug. In recombinant systems, the ϵ subunit, which is found in amygdala and thalamus and which is particularly abundant in the subthalamic nucleus, confers insensitivity of recombinant $\alpha\gamma\epsilon$ GABA_A receptors to general anaesthetics.

Neurosteroids

► **Neurosteroids** prolong the mean open time of recombinant GABA_A receptor channels. Whereas, at least in recombinant systems, the identity of the α and β subunits has little or no effect on neurosteroid action, substitution of the γ subunit by a δ subunit suppresses the GABA-modulatory activity of the neurosteroids.

GABA_A Receptor Mutants as Models for Disease

Several animal models based on the generation of targeted mutations (see also chapter “Transgenic Animal Models”) in GABA_A receptor subunits have been developed. Mice carrying point mutations in certain α subunits rendering the respective receptors GABA-sensitive but diazepam-insensitive have already been discussed in this chapter. They have provided information about pharmacological and at least indirectly also physiological roles of individual GABA_A receptor subtypes.

Mice lacking the $\gamma 2$ subunit die shortly after birth presumably due to a lack of receptor clustering. However, mice heterozygous for the $\gamma 2$ knockout allele display a limited reduction of GABA_A receptor function, visualized by decreased ligand binding and receptor clustering, notably in hippocampus and cortex. In addition, these mice display enhanced reactivity to naturally aversive stimuli such as novelty, exposed space and brightly illuminated areas, representing anxiety-related responses thought to include the activity of the septo-hippocampal system. Furthermore, the heterozygous mice display a heightened fear response in assessing the negative association of an ambiguous stimulus in cue discrimination learning; these mice perceived a partial stimulus as threatening as a fully conditioned stimulus [6]. They represent a genetic model of anxiety and represent a correlate of the GABA_A receptor deficit identified in patients with panic disorder.

Deletion of the gene encoding the $\beta 3$ subunit resulted in a loss of half of the GABA_A receptors at birth. Most homozygous knockout mice died in the perinatal period, perhaps in part due to a cleft palate, and the few survivors were interbred on a hybrid background and grew to normal body size. They displayed various neurological impairments including hyperresponsiveness to sensory stimuli, strong motor impairment and epileptic seizures, which might be due to the lack of GABA_A receptors containing the $\beta 3$ subunit that act as “desynchronizers” of neuronal activity. In these mice, the immobilizing actions of halothane and enflurane are also reduced.

Mice lacking the δ subunit, which is mainly expressed in cerebellum and thalamus, display an attenuation of ssatrighting reflex time following the administration of the neurosteroids, alphaxalone and pregnanolone, while the responses to propofol, etomidate, ketamine and the benzodiazepine midazolam were unaffected. This demonstrates the role of GABA_A receptors containing the δ subunit for neurosteroid action.

Mice lacking the $\alpha 6$ subunit display no change in the response to pentobarbital, general anaesthetics or ethanol, but are more sensitive to the motor-impairing action of diazepam in an accelerated rotarod test, though in a limited dose range only. These mice display significant compensatory changes in GABA_A receptor subunit composition and also GABA_A receptor expression levels in the forebrain. A selective posttranslational loss of the δ subunit revealed a close association of the $\alpha 6$ and δ subunits in vivo. In these mice, a compensatory upregulation of two-pore domain potassium channel, TASK-1, was found.

Mice lacking the $\alpha 1$ subunit have also been generated, as well as mice lacking the $\beta 2$ subunit. Given the fact that these are the most abundant α and β subunits respectively, it is surprising that these animals survive, presumably indicating the presence of compensatory mechanisms.

The GABA_B Receptor

The GABA_B receptor is a heterodimer of GABA_BR1 and GABA_BR2. GABA_BR1 exists in two isoforms that differ at the N-terminus, GABA_BR1a and GABA_BR1b. GABA_B receptors are coupled to second messenger systems via G proteins. Presynaptic GABA_B receptors influence neurotransmission by suppression of neurotransmitter release, presumably by inhibiting Ca²⁺ channels. Postsynaptic GABA_B receptors hyperpolarize neurons by activating an outward K⁺ current that underlies the late inhibitory postsynaptic potential (▶IPSP), presumably mediated by inwardly rectifying potassium channels of the Kir3 type.

Currently, baclofen is the only clinically used GABA_B receptor agonist. It is used as a muscle relaxant for treatment of spasticity in spinal injury and multiple sclerosis. The cloning of GABA_B receptors has renewed the interest in the search for more selective drugs and novel therapeutic indications.

- ▶Benzodiazepines
- ▶Sleep
- ▶General Anesthetics
- ▶Glycine Receptors

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Gaddum

The method of Gaddum compares equiactive concentrations of agonist in the absence and presence of a concentration of noncompetitive antagonist that depresses the maximal agonist response. These concentrations are compared in a double-reciprocal plot (or

variant thereof) to yield the equilibrium dissociation constant of the noncompetitive antagonist–receptor complex (chemical measure of the potency of the antagonist).

- ▶Drug–Receptor Interaction

Galanin

Galanin is a biologically active neuropeptide containing 30 amino acids and an unamidated C-terminus in human; galanin from other species contains 29 amino acids and C-terminal amidation.

- ▶Galanin Receptors

Galanin Receptors

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Synonyms

Galanin receptor type 1; Galanin receptor type 2; Galanin receptor type 3; Galanin receptor-1; Galanin receptor-2; Galanin receptor-3; Galanin-R1; Galanin-R2; Galanin-R3; Gal-R1; Gal-R2; Gal-R3; GalR1; GalR2; GalR3; GAL1; GAL2; GAL3

Definition

The ▶galanin receptors form a distinct subfamily of G protein-coupled receptors (GPCRs). Three ▶receptor subtypes have been cloned and characterized: GAL1, GAL2, GAL3. Structurally, each receptor is a typical ▶GPCR, i.e., a monomeric serpentine protein with seven helical transmembrane domains, an extracellularly directed N-terminus and an intracellularly directed C-terminus. Other GPCRs which are similar at the amino acid level (30–38%) include the somatostatin sst₄ and sst₅ receptor subtypes, the ORL1 (nociceptin) receptor and the GPR54 (KiSS-1) receptor. These galanin receptors are thought to mediate various effects of galanin and galanin-related peptide, such as mood regulation, cognition, pain, growth, and energy balance [1, 2, 3].

Basic Characteristics

Galanin Overview

Galanin was first described in 1983 by Tatemoto et al. as a 29-residue C-terminally amidated peptide isolated from a porcine intestinal extract. The name “galanin” was assigned to reflect the presence of glycine and alanine in the N- and C-terminal positions, respectively. The N-terminal sequence galanin-1–14 is highly conserved across species, with 100% identity for example in porcine, human, rat, and mouse. Structure-activity analyses indicate the N-terminal region is the primary peptide pharmacophore involved in receptor recognition. The C-terminus is relatively less conserved across species; human galanin is particularly distinguished in this region for terminating at position 30 with an unamidated serine.

Galanin has a widespread distribution in regions such as brain, spinal cord, and gastrointestinal tract. Galanin is linked to physiological processes such as mood regulation, neuroendocrine release, alcohol abuse, cognition, seizure, nerve growth and regeneration, food consumption, cardiovascular and gastrointestinal function. Chronic ethanol consumption increases galanin mRNA in the hypothalamus, and high levels of stress increase galanin release in the central amygdala. Transgenic mice overexpressing galanin exhibit cognitive defects resembling those seen in **▶Alzheimer’s disease**; they also display an elevated threshold for thermal **▶nociception**, suppressed seizure in response to hippocampal kindling, and elevated release of central monoamines (such as norepinephrine and serotonin) after a forced swim stress. Transgenic mice with a loss-of-function mutation in the *galanin* gene exhibit defects in nociception, spinal reflex and, nerve regeneration in various models of pain and nerve injury; they also have fewer cholinergic neurons in the basal forebrain. Female *galanin* (–/–) mice show defects in prolactin secretion and lactotroph proliferation.

Galanin Analogs

The pharmacology of the galanin receptor system was first explored using galanin peptide analogs having agonist or antagonist activity: (i) galanin peptides containing modified and/or unnatural amino acids, such as D-Trp²-galanin-1-29, (ii) peptide fragments or extensions such as galanin-1-15 or galanin-(–7-29), (iii) chimeric peptides in which the N-terminus of galanin (commonly galanin-1-13) is fused with a C-terminus comprised of a novel sequence as in the GALR2-selective agonist AR-M1896 (GAL(2-11)-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-NH₂), or a fragment of a bioactive peptide such as NPY, bradykinin, substance P (well known examples are M15, M32, M35, C7, and M40). Galnon and galmic are two nonpeptide agonist structures which have also been shown to have pharmacological activity.

Galanin-like Peptide

In 1999, Ohtaki et al. reported the purification of a novel 60-residue peptide from a fraction of porcine hypothalamic extract, on the basis of functional activity in a GAL2 receptor assay. Residues 9–21 of the 60-residue peptide shared 100% sequence identity with galanin-1-13, thereby prompting the name “galanin-like peptide,” or GALP. **▶GALP** is more discretely localized than galanin; mRNA for GALP in rat and mouse brain is limited to hypothalamus (arcuate), median eminence and pituitary neural lobe, with additional expression observed in rat gut. GALP binds and activates GAL1, GAL2, and GAL3, with highest affinity for GAL3. The corresponding receptors are perhaps more accurately described as galanin/GALP receptors.

The GAL1 Receptor

The cloned human GAL1 receptor cDNA encodes a protein of 349 amino acids. Human GAL1 shares 42% amino acid identity with human GAL2 and 38% with human GAL3 (Table 1). GAL1 receptor homologs have also been cloned from rat and mouse, with amino acid identities of 92 and 93%, respectively, relative to human (Table 1). The human *GAL1* gene on chromosome 18q23 has an unusual intron/exon organization for a GPCR, with a coding region interrupted by two introns. The mouse *GAL1* gene has been mapped to chromosome 18E4, syntenic with the human *GAL1* gene, and has a similar intron/exon organization.

The cloned human GAL1 receptor binds porcine [¹²⁵I]-[Tyr²⁶]galanin and is functionally coupled to G_i/G_o-type G proteins or related pathways. Specific examples of **▶second messenger** effects measured in vitro include reduction of forskolin-stimulated cAMP accumulation and stimulation of MAP kinase activity. The cloned GAL1 receptor activates G protein-coupled inwardly rectifying K⁺ channels (**▶GIRKs**) when transfected into *Xenopus* oocytes. Thus, native GAL1 receptors on mammalian neurons are likely to hyperpolarize and inhibit neurotransmitter release.

A pharmacological signature for GAL1 was based on rank order of binding affinity for galanin and derivatives: human galanin, rat galanin, porcine galanin > porcine galanin-1-16 > porcine galanin-2-29 > porcine D-Trp²-galanin > galanin-3-29. Among the chimeric galanin peptide constructs, the rank order of binding affinity is M32, M35, C7, M15 > M40. All peptides with measurable binding activity are agonists in vitro. The pharmacological profile is similar to that derived for the native GAL1 receptor in the Bowes melanoma cell line.

Human GAL1 receptor mRNA has been detected in multiple cell and tissue samples including Bowes melanoma cells, brain, gastrointestinal tract (from esophagus to rectum), heart, prostate, and testes. Rat GAL1 mRNA was detected in olfactory regions, many hypothalamic nuclei (including supraoptic nucleus),

Galanin Receptors. Table 1 Galanin receptor subtype relationships (% amino acid identity)

Receptor	Human GAL1	Human GAL2	Human GAL3	Rat GAL1	Rat GAL2	Rat GAL3
Human GAL1	100%					
Human GAL2	42%	100%				
Human GAL3	38%	58%	100%			
Rat GAL1	92%	41%	38%	100%		
Rat GAL2	40%	87%	58%	40%	100%	
Rat GAL3	37%	58%	92%	36%	55%	100%

Overall amino acid identity values were generated with the GAP program (GCG, Inc., Madison, USA).

amygdala, ventral hippocampal CA fields, dorsomedial thalamic areas, brainstem (medulla oblongata, locus coeruleus, and lateral parabrachial nucleus), spinal cord (dorsal horn), and pancreas-derived cells (RIN-14b). Rat GAL1 mRNA distribution and expression level were essentially constant when examined from embryonic day 20 to postnatal day 70, suggesting that GAL1 receptors in the CNS function broadly in normal synaptic transmission. Under certain circumstances, however, GAL1 mRNA is up- or downregulated, as in the following examples: (i) GAL1 mRNA was elevated in rat locus coeruleus after precipitated withdrawal from chronic morphine treatment. (ii) GAL1 mRNA was also elevated in rat hypothalamic nuclei after treatment with metabolic inhibitors (the glucose antimetabolite 2-deoxy-glucose or the fatty acid antimetabolite sodium mercaptoacetate, both of which stimulate feeding) or by salt loading. (iii) Hypothalamic GAL1 mRNA was decreased by hypophysectomy and also by lactation. (iv) Hypothalamic GAL1 mRNA was variable across the estrous cycle in female rats, and decreased in males by castration except when testosterone was administered. (v) GAL1 mRNA was decreased in the dorsal horn after inflammation or peripheral nerve injury.

The GAL1 receptor has been linked to multiple actions of galanin including feeding, nociception, neuroendocrine release, cognition, emotion, stress response, morphine withdrawal, metabolism, and gastrointestinal function. The following examples provide support: (i) In a study of rat feeding behavior, the galanin-induced feeding response was attributed either to GAL1 or a GAL1-like receptor, based on similarity between the *in vitro* pharmacological profile for GAL1 and the *in vivo* actions of various peptides when injected *i.c.v.* (Galanin produced a stronger feeding response than galanin-2-29, galanin-3-29 or galanin-1-16). (ii) In a study of the nociceptive reflex pathway in rat, intrathecal administration of a cell-penetrating peptide nucleic acid complementary to GAL1 attenuated the inhibitory effect of galanin on the flexor reflex, suggesting a role in pain processing. (iii) In an allodynic Bennett rat model, a GAL2-selective peptide AR-M1896 was inactive whereas a GAL1/GAL2-selective peptide AR-M961 increased the threshold for mechanical **▶allodynia**. Based on these

data the GAL1 receptor was considered a potential target for the treatment of neuropathic pain. (iv) In human colonic cells, GAL1 mRNA was upregulated by the inflammatory nuclear transcription factor NF- κ B and also by pathogenic *E. coli*, resulting in an increase in Cl^- secretion; thus GAL1 may be partly responsible for excessive fluid secretion during infectious diarrhea. In a separate study of guinea pig ileum, GAL1 antagonists blocked galanin-induced inhibition of electrically induced contraction. (v) In a study of rat brain cortical slices, GAL1 antagonists blocked galanin-induced inhibition of acetylcholine release, consistent with a role in cognitive function. *GAL1* (−/−) mice also show deficits in trace-cued fear conditioning but not in other cognitive tests. (vi) *GAL1* (−/−) mice also showed increased anxiety-like behavior in the elevated plus maze although they were normal in other anxiety models.

Children with the 18q- syndrome exhibit a growth hormone insufficiency phenotype and display a common 2-megabase deletion in chromosome 18q resulting in loss of the *GAL1* gene; these data suggest a possible role for GAL1 in promoting growth and development. Interestingly, human subjects with aberrations in chromosome 18q mapping to the *GAL1* gene show tumor progression and decreased survival rate for head and neck cancers. It has since been reported that GAL1 mediates an antiproliferative in oral squamous cell cancers.

The GAL2 Receptor

The cloned human GAL2 receptor cDNA encodes a protein of 387 amino acids. Human GAL2 shares 42% amino acid identity with human GAL1 and 58% with human GAL3 (Table 1). The human *GAL2* gene, located on chromosome 17q25.3, has a single intron interrupting the coding region just after TM3. The cloned rat GAL2 receptor homolog has 15 fewer amino acids in the C-terminus and shares only 87% amino acid identity with human GAL2 (Table 1). The mouse GAL2 receptor has been cloned and mapped to chromosome 11.

The cloned human GAL2 receptor binds porcine [^{125}I]-[Tyr 26]galanin and couples readily to G_q/G_{11} -type G proteins or related pathways *in vitro*. Under certain conditions, GAL2 also appears to couple with G_i/G_o - and G_{12} -type proteins [1, 2]. Specific examples of

second messenger effects measured in vitro include inositol phosphate hydrolysis, intracellular calcium mobilization, stimulation of MAP kinase activity, reduction of forskolin-stimulated cAMP accumulation, and induction of stress fiber formation. The cloned GAL2 receptor activates Ca^{++} -dependent Cl^- channels when transfected into *Xenopus* oocytes. Native GAL2 receptors in H69 small lung cell carcinoma cells are proposed to activate the monomeric GTPase RhoA, which functions in cell migration.

A pharmacological signature for GAL2 was based on rank order of binding affinity for galanin and derivatives: human galanin, rat galanin, porcine galanin, porcine galanin-2-29, porcine galanin-1-16 > porcine D-Trp²-galanin > galanin-3-29. A distinguishing feature of GAL2 is its relatively high preference for porcine galanin-2-29. Among the chimeric galanin peptide constructs, the rank order of binding affinity is M32 > M35, C7, M15, M40. All peptides with measurable binding activity are agonists in vitro.

Human GAL2 receptor mRNA has a widespread distribution in several central and peripheral tissues including hippocampus, kidney, liver, small intestine, and retina; depending on the study GAL2 mRNA has also been found in hypothalamus and pituitary. In rat brain, GAL2 mRNA was found in anterior and posterior hypothalamus (including POMC neurons of the arcuate), dentate gyrus of the hippocampus, amygdala, pyriform cortex, dentate gyrus, raphe and spinal trigeminal nuclei, mammillary nuclei, cerebellar cortex (Purkinje cells) and discrete brainstem nuclei including dorsal motor nucleus of the vagus. Interestingly, GAL2 mRNA was relatively more widespread and abundant in neonatal rat brain studied on postnatal days 0–7 than in the adult, with highest levels in neonatal neocortex and thalamus. These data suggest that GAL2 may have distinct functions related to establishment of synaptic connections in the developing brain, with implications for neural damage and repair in the adult nervous system. In rat periphery, rat GAL2 mRNA was found in vas deferens, prostate, uterus, ovary, stomach, large intestine, dorsal root ganglia, and anterior plus intermediate lobes of the pituitary as well as pancreas-derived cells (RIN-m5f).

The GAL2 receptor has been linked to multiple actions of galanin including neurotransmitter and neuroendocrine release, growth, reproduction, seizure, cognition, emotion, nociception, nerve regeneration, peripheral metabolism, and gastrointestinal motility. Support is provided by the following examples: (i) In a study of rat jejunal contraction, the galanin-dependent contractions were attributed to GAL2 based on the abundance of GAL2 mRNA in the jejunum, and on the relative efficacy of galanin-2-29 and galanin-1-16 compared to galanin and galanin-3-29. (ii) GAL2 mRNA levels are modulated by nerve injury. Three days after peripheral tissue inflammation in the rat a

peak elevation of GAL2 mRNA was observed in dorsal root ganglia. Seven days after facial nerve crush in the rat a peak elevation in GAL2 and galanin mRNA was observed in motor neurons of the ipsilateral facial nucleus. Conversely, GAL2 mRNA in dorsal root ganglia was downregulated after axotomy. (iii) In a study of normal rats, intrathecal administration of the GAL2-selective peptide AR-M1896 produced mechanical and cold allodynia; thus GAL2 was proposed to mediate sensory processing in the spinal cord. (iv) In the Morris swim maze, rats injected with galanin in the dorsal and ventral dentate gyrus (an area of GAL2 mRNA expression) displayed a significant spatial learning deficit while maintaining normal swim speed and performance, consistent with a role for GAL2 in learning and acquisition. (v) *Galanin* (–/–) mice were found to have ~30% fewer cholinergic neurons in the basal forebrain than wild type counterparts, suggesting that galanin normally exerts a trophic effect in this region. In people with Alzheimer's disease, the dwindling population of cholinergic neurons in basal forebrain is hyperinnervated by neuronal fibers expressing galanin, prompting speculation that trophic effects mediated by a GAL2-like receptor might counteract the degenerative process. (vi) The *GAL2* gene is localized on chromosome 17q25 in a region associated with two diseases (hereditary neuralgic amyotrophy and Russel-Silver syndrome) that are characterized by short stature and low birth weight dwarfism, respectively, in addition to developmental defects. This relationship suggests a possible role for GAL2 in growth and development.

GAL2 (–/–) mice were originally reported to have normal function in a broad range of tests, suggesting either compensation during development or relatively subtle contributions from the GAL2 receptor subtype. Interestingly, they did show increased anxiety-like behavior in the elevated plus maze (like *GAL1* (–/–) mice while having normal responses in other anxiety models. Moreover, in a study of pain models and mechanisms, *GAL2* (–/–) mice showed decreased neurite outgrowth in adult sensory neurons, with significant deficits in models of neuropathic and inflammatory pain, consistent with previous proposals for a role of GAL2 in sensory processing.

The GAL3 Receptor

The cloned GAL3 receptor cDNA encodes a protein containing 368 amino acids. As shown in Table 1, the human GAL3 receptor is more closely related to the human GAL2 receptor (with 58% amino acid identity) than GAL1 (with 38% amino acid identity). The human *GAL3* gene is located on chromosome 22q12.2-13.1 and has the same intron/exon organization as *GAL2*, with a single intron interrupting the coding region just after TM3. The intron/exon pattern suggests a common evolutionary origin for GAL2 and GAL3, and a convergent evolutionary relationship to GAL1. The

intron in human *GAL3* contains a Pst 1 restriction site polymorphism of unknown significance. Rat *GAL3* shares 92% amino acid identity with the human homolog (Table 1). A mouse *GAL3* homolog has been cloned and mapped to chromosome 15.

The cloned human *GAL3* receptor binds porcine [¹²⁵I]-[Tyr²⁶]galanin and is functionally coupled to G_i/G_o-type G proteins. The cloned *GAL3* receptor inhibits forskolin-stimulated cAMP accumulation. The cloned *GAL3* receptor activates GIRKs when transfected into *Xenopus* oocytes. Thus native *GAL3* receptors on mammalian neurons are likely to inhibit neurotransmitter secretion, as proposed previously for native *GAL1*.

A pharmacological signature for *GAL3* was based on binding affinity for galanin and derivatives: porcine galanin, rat galanin > human galanin, porcine galanin 2-29 > porcine galanin-1-16 > porcine D-Trp²-galanin, galanin 3-29. A distinguishing feature of the *GAL3* receptor is that human galanin binds with slightly lower affinity than rat and porcine galanin. Among the chimeric peptides, the rank order of binding affinity is M32, M35, C7 > M15, M40. All peptides with measurable binding activity are agonists in vitro. The recent availability of nonpeptide *GAL3* antagonists SNAP 37889 and SNAP 398299 provide additional tools for documenting *GAL3* responses in native cells and tissues.

Human *GAL3* mRNA was detected centrally in regions such as cerebellum, amygdala, cerebral cortex, occipital lobe, frontal lobe, temporal lobe, putamen, caudate nucleus, and spinal cord. Human *GAL3* receptor mRNA was also detected in peripheral tissues such as thyroid, adrenal gland, skeletal muscle, pancreas, gastrointestinal tract, and testes. Rat *GAL3* mRNA was found in discrete regions of the CNS such as cerebellum, amygdala, hypothalamus (ventromedial, arcuate, paraventricular, and supraoptic nuclei), olfactory pathways, cerebral cortex, hippocampus, caudate putamen, central gray, medulla oblongata, and spinal cord. Rat *GAL3* transcripts were also detected in peripheral tissues such as pituitary (a particularly rich source), liver, kidney, stomach, testes, adrenal cortex, lung, adrenal medulla, spleen, and pancreas. In general, human and rat *GAL3* transcripts were widely distributed and more overlapping with *GAL2* than *GAL1*.

The *GAL3* receptor is linked to multiple effects of galanin including mood regulation, stress response, neuroendocrine release, alcohol abuse, cognition, ception, and energy balance. Small molecule antagonists such as SNAP 37889 and close analogs produce anxiolytic- and antidepressant-like effects after in vivo administration to rodents. The various models that support this link include rat forced swim, rat social interaction and guinea pig maternal separation, among others. Mechanistic studies indicate that *GAL3* antagonists may be working in part by modulating galanin-dependent serotonergic neurotransmission in the hippocampus and dorsal raphe nucleus.

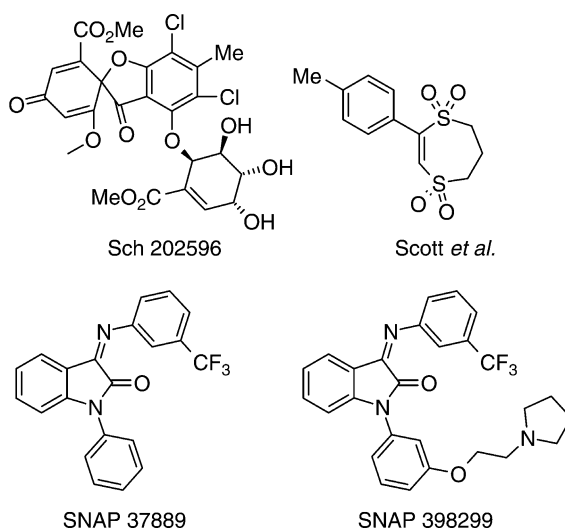
Thus *GAL3* antagonists may represent an alternate class of therapeutic agents for the treatment of depression, anxiety, and stress-related disorders.

Additional support for a role of *GAL3* in psychiatric disorder has come from analysis of chromosome aberrations and polymorphisms. The chromosomal localization of the *GAL3* gene (22q12.2-13.1) places it in a possible susceptibility locus for ►bipolar disorder (manic depressive illness). Interestingly, chromosome 22q is also a susceptibility locus for schizophrenia. Recently, a single nucleotide polymorphism in the *GAL3* gene was significantly associated with alcoholism.

Drugs

There are two descriptions of nonpeptidic ligands for galanin (*GAL1*) receptors: (i) A fungal metabolite Sch202596 (spirocoumaranone with a molecular mass of 353 D) was reported by Chu et al. in 1997 to bind the *GAL1* receptor in human Bowes melanoma cells with an IC₅₀ of 1.7 micromolar (Fig. 1). (ii) A series of 1,4-dithiins and dithiepine-1,1,4,4-tetroxides with molecular weights ~250–450 Da were reported by Scott et al. in 2000 to bind human *GAL1* in Bowes melanoma cells with affinities (IC₅₀ values) ranging from 0.19 to 2.7 micromolar (Fig. 1). Two of the dithiepinines were characterized as human *GAL1* antagonists, based on activity in a cAMP accumulation assay and also in a GTPγ³⁵S binding assay. The two dithiepinines of interest also blocked galanin-induced inhibition of acetylcholine release from rat cortical brain slices or synaptosomes, as well as galanin-induced inhibition of electrically induced contraction in the guinea pig ileum.

Significant progress has been made in the development of nonpeptidic ligands for the *GAL3* receptor (Fig. 1). 3-Arylimino-2-indolones including SNAP 37889 and SNAP 398299 were shown by Swanson



Galanin Receptors. Figure 1 Nonpeptide antagonists for *GAL1* (top) and *GAL3* (bottom).

et al. in 2006 to be potent GAL3 antagonists with binding affinity <20 nM and CNS penetrance after oral administration in preclinical models of anxiety and depression [4]. A close analog 3-(3,4-dichlorophenylimino)-1-(6-methoxypyridine-3-yl)indolin-2-one was demonstrated to have similar in vivo properties by Barr et al. in 2006 [5]. Availability of these structures may provide a way forward for elucidation of GAL3-mediated effects in humans.

► Appetite Control

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Galanin-like Peptide (GALP)

GALP is a biologically active peptide comprised of 60 residues found in human, rat, mouse, and pig. Residues 9–21 of the 60-residue peptide share 100% sequence identity with galanin-1-13, thereby prompting the name “galanin-like peptide,” or GALP. GALP is known to bind and activate GAL1, GAL2, and GAL3 receptors.

► Galanin Receptors

GAPs

GTPase Activating Proteins.

► Small GTPases

Gasotransmitters

A family of endogenous molecules of gases or gaseous signaling molecules, including NO, CO, H₂S, and others. These particular gases share many common features in their production and function but carry on their tasks in unique ways, which differ from classical signaling molecules, in the human body. To be characterized as a gasotransmitters, all of the following criteria should be met: (i) It is a small molecule of gas, (ii) It is freely permeable to membranes. As such, its effects do not rely on the cognate membrane receptors, it can have endocrine, paracrine, and autocrine effects. In their endocrine mode of action, for example, gasotransmitters can enter the blood stream, be carried to remote targets by scavengers and released there, and modulate functions of remote target cells, (iii) It is endogenously and enzymatically generated and its production is regulated, (iv) It has well-defined and specific functions at physiologically relevant concentrations. Thus, manipulating the endogenous levels of this gas evokes specific physiological changes, (v) Functions of this endogenous gas can be mimicked by its exogenously applied counterpart, and (vi) Its cellular effects may or may not be mediated by second messengers, but should have specific cellular and molecular targets.

► Carbon Monoxide

► Nitric Oxide

Gastric H,K-ATPase

The H,K-ATPase, expressed in the parietal cells of the stomach, transports H⁺ ion from cytoplasm to lumen in exchange for extracytoplasmic K⁺ ion in an electro-neutral exchange using the energy of ATP hydrolysis.

► Proton Pump Inhibitors and Acid Pump Antagonists

Gastric Inhibitory Peptide (GIP)

Synonyms

Glucose-dependent-insulinotropic peptide

► Incretin Hormones

Gastrin

Gastrin is a peptide hormone, which is synthesized in cells of the mucosa of the gastric antrum and duodenum. It is secreted into the portal blood. The main effect of gastrin is the stimulation of secretion of acid by the parietal cells of the stomach. It also increases pepsinogen secretion and stimulates blood flow and gastric motility. The release of gastrin from G-cells in the gastric antrum is controlled by various mechanisms. Vagal stimulation to the antrum causes presynaptic release of acetylcholine, which causes the release of gastrin releasing peptide (GRP) from GRP-postsynaptic neurons, which directly stimulates the endocrine release of gastrin from G-cells. G-cells are also stimulated to release gastrin in response to protein digestion products on the luminal surface. These include amino acids and small peptides, which act directly on the gastrin-secreting cells. The release of somatostatin from D-cells, which are localized in the vicinity of G-cells has a negative influence on gastrin secretion. Release of somatostatin is enhanced by high luminal acidity, which provides a negative feedback for the endocrine pathway. Release of somatostatin is inhibited by vagal cholinergic neurons. Gastrin exerts its effects by binding to a ►G-protein coupled receptor (CCK₂) on ECL cells. ECL cells respond by a release of histamine, which by activation of ►G-Protein coupled receptors (histamine receptors) causes parietal cells to secrete H⁺ ions.

Gastrin Releasing Peptide

- Bombesin-like Peptides

Gastroesophageal Reflux Disease (GERD)

Gastroesophageal reflux disease (GERD) is a digestive disorder. Gastric acid flows back up into the esophagus through the lower esophageal sphincter (LES), which connects the esophagus and stomach. The acid is irritating to the esophagus and causes heartburn.

- Proton Pump Inhibitors and Acid Pump Antagonists

Gating

Gating, a property of many ion channels, is the active transition between open and closed states in response to specific signals, such as membrane voltage or the presence of neurotransmitters.

- Voltage-dependent Na⁺ Channels
- Voltage-gated K⁺ Channels
- Voltage-dependent Ca²⁺ Channels
- Nicotinic Receptors
- Ionotropic Glutamate Receptors
- Cyclic Nucleotide-regulated Cation Channels
- Cl⁻ Channels and Cl⁻/H⁺ Exchangers
- GABA_A Receptors
- Glycin Receptors

G-CSF

Granulocyte-CSF.

- Hematopoietic Growth Factors

GDI

Guanine Nucleotide Dissociation Inhibitors.

- Small GTPases

GEFs

Guanine Nucleotide Exchange Factors.

- Small GTPases

Gene Activity Profile

A gene activity profile is a collection of quantitatively determined levels of gene products, found in one tissue or cell type, which is characteristic of the tissue, a disease process, a hormone response, a pharmaceutical intervention, etc.

- Gene Expression Analysis
- Microarray Technology

Gene Array

- Gene Expression Analysis
- Microarray Technology

Gene Chip

- Gene Expression Analysis
- Microarray Technology

Gene Expression Analysis

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Synonyms

Gene chip technology; Transcriptome analysis

Definition

DNA ►microarrays, or DNA chips consist of thousands of individual DNA sequences arrayed at a high density on a single matrix, usually glass slides or quartz wafers, but sometimes on nylon substrates. Probes with known identity are used to determine complementary binding, thus allowing the analysis of ►gene expression, DNA sequence variation or protein levels in a highly parallel format.

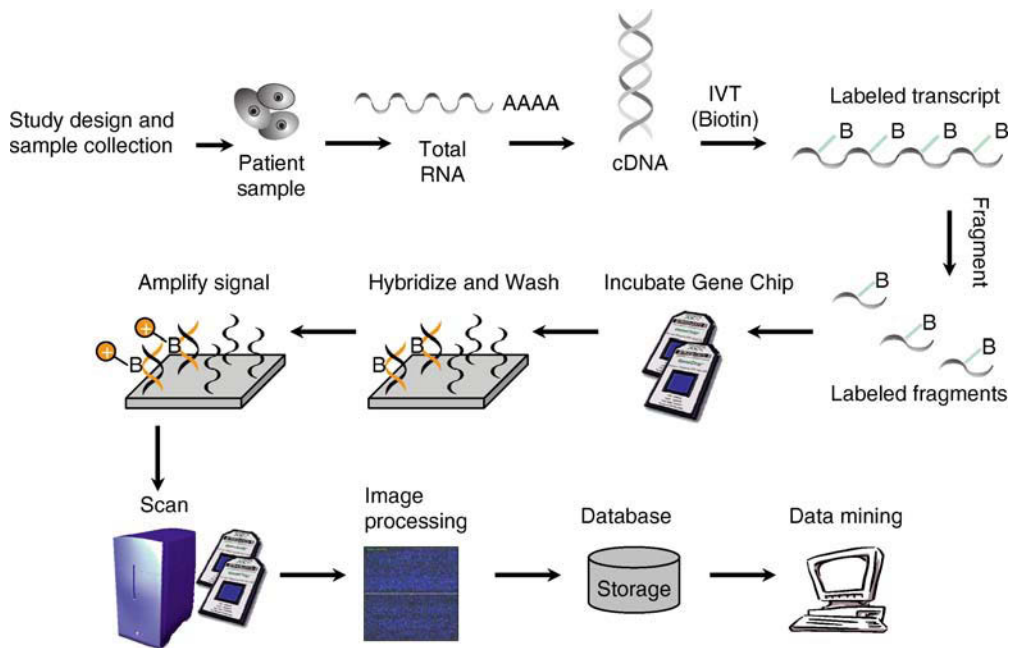
Description

High throughput technologies, like gene expression microarray are established to assess the activity status of genes in a highly parallel manner, i.e. synchronous analysis of the entire ►‘transcriptome’ at the level of messenger RNA. DNA microarray technology is a multi-step process: (i) design and sample collection, (ii) preparation and labelling of RNA/cDNA,

(iii) hybridization to a selected microarray platform, (iv) scanning of microarrays, (v) statistical analysis, (vi) biological interpretation and validation of the results. Many factors influencing the microarray experiment and the variability at each step must be reduced to maximize the probability of uncovering biological knowledge. Careful experimental design of the microarray will ensure the maximal potential gain in efficiency and is particularly important if the resulting experiment is to be maximally informative, given the effort and the resources.

There are many protocols and different types of platforms available, e.g. GeneChips from Affymetrix, Illumina Bead Arrays, Arrays from Agilent, Applied Biosystems, GE Healthcare, customized spotted cDNA microarrays etc.; the basic procedure for a large-scale measurement of gene expression involves the preparation of total or mRNA from the biological sample(s) under investigation (e.g. ‘candidate’ tissue) and the hybridization of copied ‘labelled’ RNA or cDNA to the DNA elements on the array surface (Fig. 1).

To ensure a high reproducibility, fluctuations in sample preparation and hybridization need to be reduced to a minimum. Major sources of random fluctuations to be expected are in probe, target and array preparation, e.g. in mRNA preparation, ►reverse transcription, labelling, target volume, hybridization parameters, overshining effects, non-specific background, variations in pin geometry during spotting of cDNA, slide inhomogeneities and image analysis. Replicates of each experiment should be used in order to reduce variability and to differentiate between experimental variation and real expression differences. Suitable internal controls ensure quality control measurements for samples and array. After the hybridization process, intensity values from the hybridized RNA samples are detected by phospho-imaging or fluorescence scanning and independent images are generated. Then background intensity is subtracted and signal intensities are usually normalized to compensate for experimental variability and to ‘balance’ the signals from the two samples being compared. All normalization techniques assume that all or a subset of spots (e.g. genes) on the array have an average expression ratio equal to one. The normalization factor is then used to adjust the data (signal intensities) from the two samples and to ensure that the total quantity of RNA hybridized to the array is the same. Mean spot or transcript intensities are calculated and ratios of intensities are used to account for relative expression differences. In a simple pairwise comparison of gene expression between two samples, the results can be shown in plots of the intensities or the log of the intensity ratios. Scatter plots are widely used to make the observed differential expression visible. For further details please see [1].



Gene Expression Analysis. Figure 1 Schematic representation of the experimental process of global gene expression analysis using Affymetrix GeneChip arrays.

Currently, there is a lack of widely accepted [QA/QC control metrics](#) for DNA microarray technologies, and there is currently no consensus on how to establish the reliability of the results obtained from a DNA microarray experiment (for more information please check MicroArray Quality Control (MAQC) project, [2]) QA/QC pass/fail filters to eliminate outlier arrays are used by some companies and organizations, and some array manufacturers recommend thresholds for certain platform-specific QC measurements. It might be useful to develop [spike-ins](#) and reference standards to evaluate the quality of a particular microarray experiment in the future. Another recent effort has produced a pair of reference RNAs for use with rat DNA microarrays that allows accuracy, reproducibility and dynamic range assessments [3]. Conceptually, this strategy could be used to produce reference materials for any organism, including human. Until such independent resources are widely available and consensus quality standards are developed and implemented by the microarray community, carefully adhering to the microarray manufacturer's recommended procedures offers the best current practice at this time. Because the microarray field is an evolving field, it is important to note that manufacturers occasionally change probe sequences and protocols, reflecting continuing improvements to this technology.

After the hybridization process, intensity signals from the hybridized RNA/cDNA samples are usually detected by phospho-imaging or fluorescence scanning and independent images are generated.

Microarray experiments generate large and complex data sets that constitute e.g. lists of spot intensities and intensity ratios. Basically, the data obtained from microarray experiments provide information on the relative expression of genes corresponding to the mRNA sample of interest. Computational and statistical tools are required to analyze the large amount of data to address biological questions. To this end, a variety of analytical platforms are available, either free on the Web or via purchase of a commercially available product.

Once the list of statistical significant de-regulated genes (De-regulated gene in the context of gene expression analysis usually means that the mRNA amount of a certain gene is different in the compared samples.) has been generated, the next step in the process is to interpret the biological meaning of gene expression changes and determine whether biological pathways may be of functional relevance to the mechanism of drug action, or maybe correlated to safety and/or efficacy.

A number of points should be addressed, e.g.:

- Are genes from a particular pathway or set of pathways significantly overrepresented in the list?
- How many pathways are affected?
- Can the mechanism of action be inferred from the functions of the pathways altered or from the pattern of expression across the genes within these pathways?
- What is the tissue specificity of the pathways and the gene function in relation to biological processes?

- What are the magnitude and/or pattern of the alteration in a particular pathway in relation to treatments with other compounds (related or unrelated) with known pharmacological or toxicological properties?

At present, no single tool can be used to find answers to all these questions, but a combination of tools can be used to address a particular question of interest as thoroughly as currently possible.

An overlap of the biological interpretations obtained with two or more different databases can facilitate a consensus on what the interpretation should be. However, this is not always the case. Consensus can be hindered by many factors including, but not limited to, absence of information on the compound of interest in the reference databases or a lack of annotation for particular pathways of interest. For example, subsets of genes may be placed in specific pathways in one system, but they may not be represented in the same pathways in another pathway analysis tool, or genes may not have been evaluated in a particular platform. In pathway analysis databases, the information may differ depending on which content is extracted from the literature and how that extraction is performed. A heavy reliance on the literature and on reference databases is recommended to extract functional information on specific gene lists and generate hypotheses on the biological significance of the relevant set of genes.

Pharmacological Relevance

Genomics, Proteomics and related disciplines that make use of systematic and highly parallel analyses of biological parameters serve support functions in several steps of the drug discovery process. Traditionally, the central focus in pharmaceutical research has been on target identification, since it had been expected that the discovery of novel genes involved in disease will help to discover new, effective and proprietary disease treatments. However, an abundance of potential target genes for many human diseases is publicly known as yet, putting the need to discover additional candidate genes into perspective.

Drugs currently on the market target a relatively small number of gene families, termed the 'druggable genome', dominated by the G protein-coupled receptor, kinase, protease, ion channel and nuclear hormone receptor gene families [4]. One strategy to identify novel drug targets is to mine the human genome for new members of the established families of drug targets. Such gene identification efforts based on homology searches can then be integrated with other approaches (e.g. microarray studies) to accumulate evidence that the presumed drug target is indeed a disease-modifying gene product. Alternatively, microarrays can be used *ab initio* to identify genes associated with pathology.

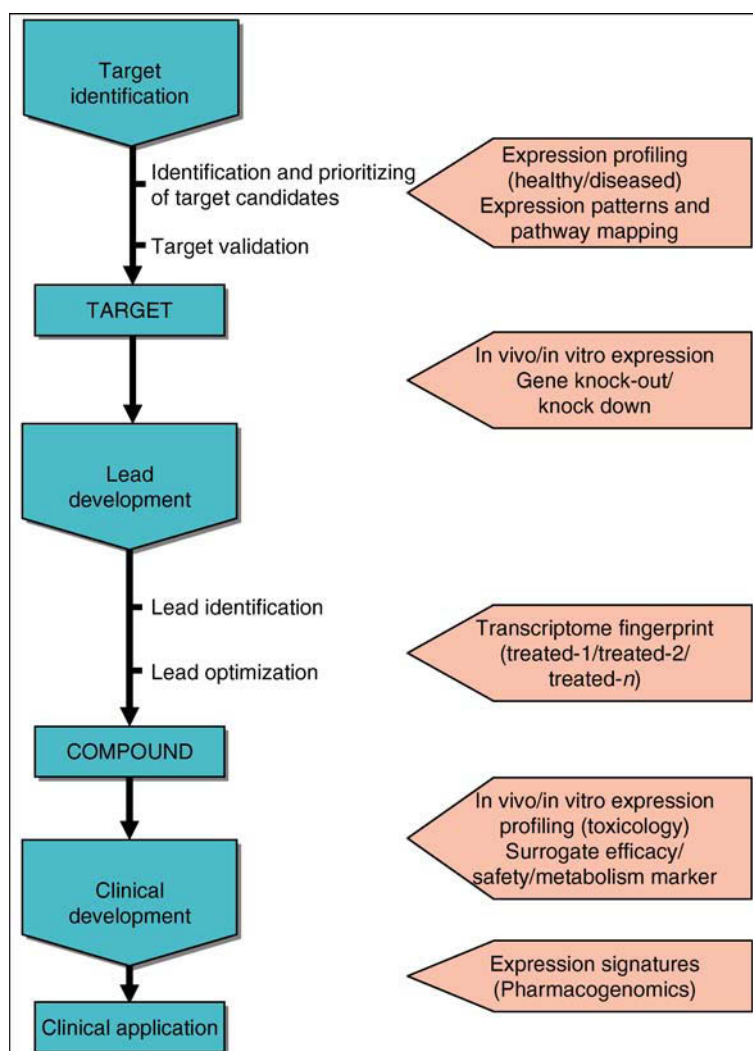
This strategy may also be used to implicate genes of unknown annotation or function in a disease process. Although microarrays are expected to have the potential to identify novel drug targets, the quality of the resulting candidate drug target is currently not easy to define. In addition, it should be looked at if the members of druggable gene families are properly represented in the [▶transcriptome](#) and/or if they are poorly expressed and below the detection limit of the current technology.

As a result of further technological improvements and decreasing costs, the use of microarrays will become an essential and potentially routine tool in understanding the mechanistic basis of action of many drugs.

The information about gene expression, global transcriptional changes induced by a particular drug and sequence variation might have an impact not only on therapeutic target identification, but also on many aspects of the drug discovery process and on drug efficacy. Microarrays are used at the following specified steps in the process of drug development:

- Target discovery, to identify genes or pathways with altered expression in diseased human tissues or in animal model of disease
- Target validation, to determine that a gene product is causative of disease symptoms or that activation of the target protein ameliorates disease symptoms. Agonist/activator or an inhibitor which may be therapeutic could be identified using microarrays
- [▶Compound optimization](#), to screen a series of therapeutic drug candidates to find the compounds that are most specific for the target protein and those that cause unintended effects, i.e. improved understanding of the molecular mode of action including structure-activity relationships for on-target versus off-target effects
- Toxicology ([▶toxicogenomics](#)), to identify potential human and environmental toxicants, and to find correlations between toxic responses to toxicants and changes in the genetic profiles of the objects exposed to such toxicants
- [▶Drug metabolism](#), to predict whether a drug candidate will cause drug-drug interactions
- [▶Drug efficacy](#), to identify the individual mode of action or adverse effects of a given drug, including identification of genes involved in conferring drug sensitivity and resistance
- Discovery of new diagnostic and prognostic indicators and [▶biomarkers](#) of therapeutic response, and prediction of patients most likely to benefit from the drug and use in general pharmacogenomic studies (Fig. 2)

At present, the use of microarrays in pharmacology is especially established in the field of oncology; by gene expression analysis with either tumour cell



Gene Expression Analysis. Figure 2 Contribution of gene expression profiling to the drug discovery process.

model systems or tumour tissue, mediators and mechanisms of drug response have been identified. One recent example is the use of in vitro drug sensitivity data coupled with Affymetrix microarray data to develop gene expression signatures that predict sensitivity to individual chemotherapeutic drugs, e.g. expression-based predictor of sensitivity to docetaxel [5]. Many of these signatures can accurately predict clinical response in individuals treated with these drugs. The integration of the chemotherapy response signatures with signatures of oncogenic pathway deregulation has led to the identification of new therapeutic strategies that make use of all available drugs. The development of gene expression profiles that can predict response to commonly used cytotoxic agents provides opportunities to better use these drugs, including using them in combination with existing targeted therapies.

- [Microarray Technology](#)
- [Pharmacogenomics](#)
- [Proteomics](#)

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Gene Gun

A gene gun is a device to introduce DNA into cells *in vivo*. The DNA is attached to gold particles, which are introduced under high velocity into the target tissue.

- DNA Vaccination and Genetic Vaccination
- Gene-therapy Vectors

Gene Products

In the context of ► [gene expression analysis](#), gene products include proximal products, such as primary transcripts, intermediate products, such as mRNA, tRNA, and rRNA, and distal products including proteins and peptides.

Gene Promoter

The gene promoter is a nucleotide sequence in DNA near the start of a gene, consisting of regulatory elements to which transcription factors and RNA polymerase bind. This leads to activation of the gene promoter and transcription of the corresponding gene.

- Transcriptional Regulation

Gene Therapy

- Gene-therapy Vectors

Gene-therapy Vectors

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Synonyms

Viral vectors; Gene transfer; Gene therapy

Definition

The basic principle of gene therapy is rather simple: therapeutic genes are transferred into the patient's cell and organs. The therapeutic effect is either achieved by correction of a genetic defect (e.g., single gene defects) or by expressing genes that positively affect disease course, patient status, and outcome. The vehicle used to transfer the therapeutic gene is still a major factor for the success or failure of gene-therapy trials.

Description

With the progress of the human genome project and functional genomics, the number of genes that could have a therapeutic impact is increasing at an astonishing pace. However, a major problem for successful gene therapy is the gene delivery vehicle.

Design of Viral Vectors

Viral vector design focuses mainly on the efficacy of gene delivery and on the biosafety of the engineered viruses. A prerequisite for the use of a virus as gene-therapy vehicle is to identify and eliminate pathological or toxic viral genes. Ideally, all viral genes are replaced by the gene of interest (also called transgene) (Fig. 1). The viral gene products required for the assembly of infectious particles and packaging of the vector into these particles are provided in *trans* by the so-called packaging cells, while necessary *cis*-acting factors like packaging signals are incorporated into the vector genome.

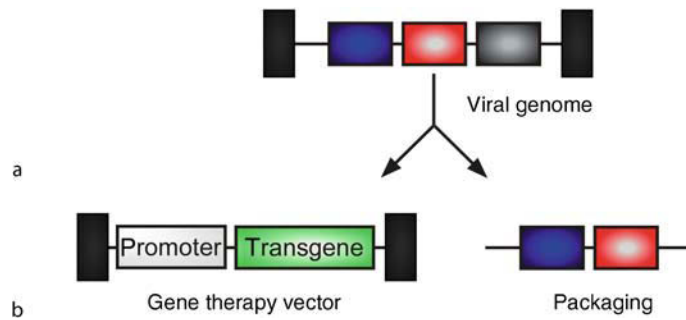
Regulation of Gene Expression

An important issue is the regulation of gene expression in the target cells and tissues. Although steady-state expression over prolonged time intervals is desired in many diseases, controlled expression of the foreign gene in a reversible manner will be highly desirable (e.g., gene therapy for insulin-dependent diabetes mellitus). To achieve more physiological expression profiles, novel regulatable systems that are controlled by endogenous (e.g., hormones) and/or exogenous (e.g., pharmacological agents) factors have to be developed. In addition, regulation of transgene expression by the gene product itself via negative or positive feedback mechanisms would be an additional desired feature.

Viral vectors are usually classified by the characteristics of the parental virus. Based on the viral genome, one can distinguish between DNA and RNA viruses (for details see [1, 2]).

DNA Virus Vectors

The most widely used DNA virus vectors are derived from adenovirus (Ad) and adeno-associated virus (AAV). Adenoviruses contain a 36-kb double-stranded DNA genome and can infect a broad spectrum of cells, including nondividing cells like hepatocytes and neuronal cells. Ad vectors can be produced at high



Gene-therapy Vectors. Figure 1 Design of gene-therapy vectors. (a) Schematic drawing of the genome of the parental virus. (b) The viral genes required for the production of infectious particles are provided *in trans* (right), the vector itself carries the gene of interest (transgene) under the control of a specific promoter.

titers ($\sim 10^{14}$ particle/ml) and can efficiently express transgenes in many different cells and tissues. However, transgene expression is only short-lived, because the Ad chromosome does not integrate into the host genome and is maintained as an episome. In addition, adenoviral infection causes a humoral and cellular immune response in immune-competent hosts. This immune response is directed not only against the viral particles but also against the infected cells. An unbalanced, massive immune response directed against Ad can be life-threatening to the patient especially if a high concentration of Ad vectors is administered [2]. Therefore, gutless Ad vectors that do not contain viral genes have been developed. Transgene expression from these gutless vectors has been reported to be more long-lasting and the host immune response less pronounced (see [1]). Replication-competent Ad vectors are especially promising for oncolytic therapy. To this end, Ad vectors, which preferentially replicate in tumor cells, have been designed.

AAVs are parvoviruses that are nonpathogenic to humans and carry a small genome of only 4.7 kb. Entry of AAV into the cell is mediated by binding to heparan sulfate proteoglycan. Integrin $\alpha v \beta 5$ and the fibroblast growth-factor receptor 1 act as coreceptors. In the absence of the so-called helper viruses (Ad and herpes viruses), AAV integrates into the host genome (human chromosome 19) and establishes latent infection. The integration of wild-type AAV is site-specific and requires the presence of the viral Rep protein, which is normally absent from AAV vectors. Therefore, recombinant AAV (rAAV) lacking the *rep* gene exhibits significantly lower integration frequencies and site-specific integration is lost. Nevertheless, long-term transgene expression can be achieved with rAAV, because extrachromosomal rAAV genomes can persist as episomes [1].

The development of AAV-based gene-therapy vectors is presently focused on the analysis of the properties of the different AAV serotypes, especially the host range of the virion shells of the different serotypes. By packaging

the AAV genome, e.g., from AAV-2 into the capsid of other AAV serotypes (crosspackaging), the tropism of AAV vectors can be changed. Crosspackaging can achieve efficient and specific transduction of target cells and tissues.

Another important issue is the size-limitation of AAV vectors: the maximum size of rAAV genomes is ~ 4.7 kb. To overcome this size constraint, dual vector systems with split-genomes have been developed [1]: dual vectors are based on episomal circular multimers formed by the AAV vector genomes. However, the efficacy of the dual vector system has been questioned.

Presently, 25% and 4% of all clinical gene-therapy trials use Ad and AAV vectors, respectively, as gene-delivery vehicle (Table 1). Although no pathology is known to be associated with AAV, the high frequency of antibodies against AAV (80% of the human population are seropositive for the most common AAV subtype) could be a major limitation for gene therapy using AAV-based vectors [3].

RNA Virus Vectors

The most commonly used RNA viruses are based on *Retroviridae*. Retroviruses are enveloped viruses that integrate into the host genome. All retroviruses contain a basic set of three genes: *gag* (the structural virion proteins), *pol* (essential viral enzymes), and *env* (the viral glycoproteins of the envelope). Prototypic retroviruses like murine leukemia virus (MLV) carry only this simple set of genes, while complex retroviruses like the lentiviruses carry additional regulatory genes. The first gene-therapy vectors were derived from prototypic retroviruses, and they were the first viral vectors to be used in clinical trials. Retroviral virions can accommodate ~ 7 kb of RNA and after reverse transcription in the target cells, the vector DNA is randomly integrated into the host chromosomes. Integration of the vector genome is of advantage if long-term (even life-long) vector expression is wanted. However, vector integration harbors the risk of insertional mutagenesis and

Gene-therapy Vectors. Table 1 Gene therapy – clinical trials

Vector	Number (of viral gene transfer protocols)
Adenovirus	25% (35%)
AAV	4% (5%)
Retrovirus	23% (32%)
Lentivirus	1% (1%)

Detailed information can be found at (► <http://www.wiley.co.uk/genetherapy/clinical/> and ► www4.od.nih.gov/oba/rdna.htm).

activation of proto-oncogenes. To cope with the latter problem, self-inactivating retroviral vectors have been developed which carry deletions of the essential viral promoter/enhancer sequences. However, incorporation of the so-called SIN (self-inactivating) mutations can result in a significant reduction of retroviral titers [1, 2].

A major drawback of vectors derived from prototypic retroviruses is that they can only transduce dividing cells. Therefore, these vectors cannot be used for gene transfer in many nondividing cells (e.g., muscle and brain cells).

To circumvent this problem, vectors that are based on lentiviruses have been developed. In contrast to prototypic retroviruses, lentiviruses do not require cell division for integration. Gene-therapy vectors have been developed from a broad spectrum of lentiviruses including human immunodeficiency virus (HIV), simian and feline immunodeficiency virus as well as visna/maedi virus. The most widely used lentiviral vector system is based on HIV-1. These vectors can efficiently transduce a broad spectrum of dividing and nondividing cells including neurons, hepatocytes, muscle cells, and hematopoietic stem cells [1, 2].

To address biosafety concerns, self-inactivating lentiviral vectors and stable packaging cell lines have been developed.

Apart from being a promising tool for gene therapy, lentiviral vectors are also important tools for molecular biology. These vectors efficiently transduce a variety of nondividing cells in vitro. Lentiviral vectors can be used to generate clinically relevant transgenic animal models (lentiviral transgenesis) (for a detailed review see [4]). Importantly, lentiviral transgenesis is not restricted to rodents (mouse and rat), but can also be used to generate transgenic animals in medically relevant livestock species (pig, cattle, chicken).

Finally, lentiviral vectors have been shown to transduce human embryonic stem cells. Therefore, this type of gene-therapy vector might also be used in stem cell-based therapies.

Retrovirus- and lentivirus-derived vectors are used in approximately one quarter of all gene-therapy trials (Table 1). The gene-therapy trial in patients suffering

from severe combined immunodeficiency-X1 (SCID-X1) led by Alain Fischer and colleagues initially reported the successful restoration of the immune systems of SCID patients [2]. In this trial, hematopoietic stem cells were transduced with vectors derived from simple retroviruses. Unfortunately, 3 years after the start of the trial, two of the patients developed leukemia. Detailed analysis of vector-integration sites revealed that the retroviral vector had integrated close to the protooncogene LMO2 [2]. These results clearly underline the risk of insertional mutagenesis inherent to gamma retroviruses (also known as oncoretroviruses, like MLV). Although lentivectors presently account for only less than 1% of the gene-therapy trials, the first publication of a clinical trial using a lentiviral vector in the *Proceedings of the National Academy of Sciences* [5] highlighted the potential of this vector system. This phase I trial evaluated the safety of a conditionally replicating HIV-derived vector expressing an antisense gene against the HIV envelope. Importantly, the study demonstrated not only safe and efficient gene delivery to the patients' T cells, but also no adverse clinical effects were detected during the follow-up for 2 years. Based on the encouraging results, a multicenter phase II trial was started (details can be found at ► <http://www.virxsys.com>).

Pharmacological Relevance

Although pharmacology still relies heavily on small chemical substances, biologicals like recombinant proteins, cell-based therapies, and gene-therapy approaches have great potential for the treatment of broad spectrum of diseases. Therefore, the focus of modern pharmacology should shift towards these innovative therapies.

Presently, 1,260 gene-therapy trials are underway worldwide (Tables 1 and 2). For details see ► <http://www.wiley.co.uk/genetherapy/clinical/>. Almost three fourths of all trials are based on viral vectors. The vast majority of nonviral gene-therapy trials use naked/plasmid DNA (18% of all gene-therapy trials).

At the present time, almost two thirds of all gene-therapy trials target immunological and oncological diseases (Table 2).

Potential Risks

An important safety issue of viral vectors is whether or not the recombinant viruses are able to replicate in the infected cells. Replication of viral vectors is unwanted in most gene-therapy approaches. Therefore, replication-defective vectors have been designed, which are able to perform only one initial infectious cycle within the target cell. In addition, replication-competent vectors have been designed, which are able to productively infect the target cell and to spread in the target tissue.

Immunological reactions of the host to either the delivery vehicle or its cargo are another concern. The host immune response might not only eliminate the vector

Gene-therapy Vectors. Table 2 List of most common therapeutic genes used in gene-therapy trials (viral and nonviral vectors)

Gene type	Number
Cytokine	27%
Antigen	16%
Tumor suppressor	11%
Suicide	8%

Detailed information can be found at (► www.wiley.co.uk/genetherapy/clinical/ and ► www4.od.nih.gov/oba/rdna.htm).

particles before they reach the target cells/tissues. In addition, it can also be directed against the product(s) of the genes delivered by the vector, especially if a null-mutation is replaced with a functional copy of the affected gene (in this case no immunological tolerance would exist for the product of the normal gene).

The development of leukemia in patients receiving retrovirally transduced cells, clearly underlines that insertional mutagenesis is a major concern for integrating vectors.

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Gene Transfer

► Gene-therapy Vectors

Gene Transfer Mechanisms (Bacteria)

Genetic material can be transferred horizontally between bacterial cells either as free DNA by transformation or as plasmid DNA via conjugational

mating of bacterial cells or as fragments of chromosomal DNA with the help of bacteriophages. Some of these virus-like particles called transducing bacteriophages can incorporate fragments of chromosomal DNA of the bacterial cell into the bacteriophage. During the course of a second infection this foreign DNA instead of bacteriophage DNA is delivered to another cell and introduced into the respective locus within the chromosome by homologous recombination.

► Bacterial Resistance to Antibiotics

General Anaesthetics

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Synonyms

Anaesthetic

Definition

General anaesthesia can encompass several different end points, the most critical of which can be defined as unconsciousness. A loss of sensation including that of any painful stimulus, and muscle relaxation are also desirable endpoints of general anaesthesia. Modern-day anaesthesia is accomplished using a balanced combination of different drugs to confer these different endpoints.

Mechanism of Action

General anaesthetics have been in use for the last 100 years, yet their mechanism of action are still not yet clearly defined. For many years it was thought that general anaesthetics exerted their effects by dissolving in cell membranes and perturbing the lipid environment in a non-specific manner. This theory derived from the observation that for a number of drugs which induced anaesthesia, their potency correlated with their oil–water partition coefficients. This Meyer–Overton correlation was accepted for a number of years, however in the last 15–20 years evidence has shown that a more likely theory is that of specific interactions of anaesthetics with proteins, particularly those within the CNS that mediate neurotransmission [1].

Two methods of anaesthesia are currently in use, the application of inhaled gaseous or volatile anaesthetics such as halothane, sevoflurane and isoflurane to maintain a level of anaesthesia. Older compounds in this category include nitrous oxide and chloroform.

The other method used is infusion of intravenous anaesthetics such as propofol, etomidate (for induction) and the barbiturates such as thiopental and pentobarbital. Investigations into the mechanism of anaesthesia have made use of all these compounds in order to identify a common mode of action linked to likely mechanisms within the CNS.

Research on anaesthetic mechanisms over the last 20 years has moved away from the lipid theory and focussed on specific protein interactions. The discovery that anaesthetics have major effects on receptors and channels within the nervous system have provided a clear and rational approach to understanding how anaesthesia is generated. Effects on ligand-gated ion channels currently offer the most likely mechanism of action, however some anaesthetics interact directly with voltage-gated channels [2]. In general, activity at voltage-gated ion channels are relatively weak and may not correlate with anaesthetic doses. Similarly, there have been some reports of interactions with G-protein coupled receptors but again at non-clinical concentrations.

Which of the ligand-gated receptors are affected by anaesthetics and are relevant in conferring unconsciousness and loss of sensation? Volatile anaesthetics and alcohols are quite promiscuous in their effects on ligand-gated channels, showing potentiation of inhibitory channels such as ►GABA_A and ►glycine receptors and inhibition of neuronal ►nicotinic, AMPA and ►NMDA receptors [3]. Where investigated these compounds also potentiate kainate and 5-HT₃ receptors (alcohols inhibit kainate). Intravenous agents are slightly more selective, potentiating GABA_A and inhibiting neuronal nicotinic receptors, however, effects on other ion channels are detectable at non-clinical concentrations. Ketamine is unique in having little effect on GABA receptors but strong inhibition of NMDA receptors. Recent studies using enantiomeric isomers of etomidate have suggested that the inhibition of nicotinic receptors is not what underlies the anaesthetic properties of this compound, but these effects are much more likely to be via potentiation of ►GABA_A receptors, a feature common to the majority of anaesthetic agents at clinically relevant concentrations. The potency of volatile anaesthetics is expressed as MAC (minimum alveolar concentration) and when expressed as aqueous concentration ranges from 0.2 to 30 mM in terms of plasma concentration. These values equate well with their potency at GABA_A receptors. Intravenous anaesthetic levels are estimated from measured drug concentrations in plasma during anaesthesia and range between 0.3 and 50 µM, however, this may underestimate the true receptor occupancy with the drug.

Many anaesthetics exist as enantiomeric pairs which when separated show selectivity in terms of anaesthetic potency. Studies on the action of anaesthetics at GABA_A receptors have mimicked this selectivity, again

providing evidence that anaesthetic effects are mediated via these receptors. The inhibitory component of all central nervous system transmission is primarily determined by GABA_A receptors, being present at most inhibitory synapses and on the majority of neuronal cell bodies. It is clear that as GABA is such a major inhibitory component, enhancement of GABAergic function will produce pronounced depression of neuronal activity, consistent with that observed during anaesthesia.

A considerable body of data now exists demonstrating that the majority of volatile and intravenous anaesthetics potentiate GABA_A receptor transmission both in vivo and in vitro. GABA_A receptors comprise of a number of subtypes dependent on the components of a pentameric arrangement of subunits, which combine to form an ion channel selectively permeable to chloride. These are made up of $\alpha, \gamma, \delta, \epsilon$ and θ , with the majority of receptors comprising of 2 α , 2 γ and a $\gamma 2$ subunit. While the subunits all have unique regional distribution the majority of these receptors show sensitivity to anaesthetic agents. One exception to this is etomidate which demonstrates receptor selectivity for those containing a $\gamma 2$ or $\gamma 3$ subunit with little effect at $\gamma 1$ containing receptors. Recent studies combining molecular biology and electrophysiology have addressed the question of the site of action of anaesthetics on the receptor and these have revealed that potentiation by volatile anaesthetics and alcohols are dramatically affected by specific mutations at a serine residue within the second ►transmembrane domain of the receptor [4]. A second residue, an asparagine in the third transmembrane domain, can also abolish effects of these agents when mutated. Further studies based on these residues have shown that the anaesthetic cut-off for receptor potentiation can be affected and that photoactivatable anaesthetics can covalently label these residues, suggesting that there may be a binding pocket for these agents within this region of the receptor. Interestingly, the majority of intravenous agents remain unaffected by these mutations indicating that a separate region is involved in the binding of non-volatile agents. The availability of GABA_A receptor subunit knockout mice, and the application of transgenic technology to generate mice containing receptor mutants such as those described above will considerably advance our understanding of anaesthetic mechanisms in the next few years.

Clinical Use (Including Side Effects)

General anaesthetics are administered for many surgical procedures where the patient is likely to undergo a severely painful procedure, and complete unconsciousness and immobility is required for the surgery to be performed. The most commonly used volatile anaesthetics are halothane, isoflurane and sevoflurane. Nitrous oxide is also commonly used, particularly during

childbirth. Side effects that may be encountered following administration of these agents are cardiovascular and respiratory depression, post operative nausea and vomiting, hepatotoxicity via metabolite induction of liver enzymes, and occasionally nephrotoxicity from breakdown products.

Intravenous general anaesthetics are becoming increasingly popular due to the ease of application and continuous monitoring. Their rapid recovery has resulted in an increased use of these drugs in ambulatory surgery. They currently represent at least 50% of the total anaesthetic market and are dominated by propofol, followed by thiopental, etomidate and ketamine. A growing method of applying these anaesthetics is by target controlled infusion. This apparatus pumps a continuous amount of drug into the blood, allowing the anaesthetist to set a desired plasma concentration, which the software inside the pump produces rapidly, but safely, by automatically controlling the infusion rate according to a continuous measure of either level of compound in the plasma or depth of anaesthesia, continuously monitored by EEG. This method known as TIVA (total intravenous anaesthesia) is becoming popular due to the ease of use and additional level of control it allows. Side effects associated with intravenous anaesthetics are less than the volatiles, particularly in regard to postoperative nausea and vomiting (especially propofol), however, cardiovascular and respiratory depression still cause problems with propofol and the barbiturates. Lack of cardiovascular side effects make etomidate particularly attractive for patients with a compromised heart condition, however, continuous infusion results in cortisol inhibition and adrenal failure, so etomidate can only be used for anaesthetic induction.

- ▶ GABAergic System
- ▶ Glycine Receptors
- ▶ Ionotropic Glutamate Receptors
- ▶ Nicotinic Receptors
- ▶ Local Anaesthetics

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General Transcription Factors

General or basic transcription factors are required for every gene to allow the proper recruitment of RNA polymerases to ensure transcriptional activity. They bind to core promoters in the vicinity of transcriptional start sites in a sequential manner.

- ▶ Transcriptional Regulation

Genetic Polymorphism

A genetic polymorphism is a difference in DNA sequence that has a frequency of at least 1% in a population. Some DNA sequence differences change the expression or function of drug metabolizing enzymes or of drug transporters or of drug target proteins and can therefore affect the disposition and action of drugs and xenobiotics. These are called pharmacogenetic polymorphisms.

- ▶ Single-Nucleotide Polymorphism
- ▶ P450 Mono-Oxygenase System

Genome-wide Association Study (GWA Study)

A study that investigates the statistical associations between a phenotype and a very large number of genetic markers supposed to inform on the global variability of the genome. Because GWA studies do not rely on a priori knowledge, they may lead to the discovery of new causes of disease.

- ▶ Pharmacogenomics

Genomics

The goal of genomics is to determine the complete DNA sequence for all the genetic material contained in an organism's complete genome.

Functional genomics (sometimes referred to as functional proteomics) aims at determining the function of the proteome (the protein complement encoded by an organism's entire genome). It expands the scope of biological investigation from studying single genes or proteins to studying all genes or proteins at once in a systematic fashion, using large-scale experimental methodologies combined with statistical analysis of the results.

Structural genomics is the systematic effort to gain a complete structural description of a defined set of molecules, ultimately for an organism's entire proteome. Structural genomics projects apply X-ray crystallography and NMR spectroscopy in a high-throughput manner.

- Bioinformatics
- Gene Expression Analysis
- Microarray Technology
- Proteomics
- Pharmacogenomics
- Pharmacogenetics

Genotype

The genetic constitution of an organism, which it has inherited from its parents. The genotype refers to the particular combination of alleles at specified loci present in an organism; homozygous individuals have identical alleles and heterozygous individuals have different alleles.

- Pharmacogenomics

Gephyrin

Gephyrin is an intracellular membrane trafficking protein implicated in plasma membrane targeting and clustering of inhibitory glycine and GABA_A receptors. The tubulin-binding protein forms oligomers, generating a submembranous scaffold at the postsynaptic face of inhibitory synapses. This scaffold serves as an anchor to the inhibitory glycine receptor and subtypes of the ►GABA_A receptor, preventing the receptor complexes from lateral diffusion. Additional components of the postsynaptic protein scaffold include the phosphatidylinositol 3,4,5-trisphosphate binding

proteins collybistin and profilin. Serving dual functions, gephyrin also contributes to the biosynthesis of the molybdenum cofactor, an essential coenzyme of dehydrogenases.

- Glycine Receptors
- GABAergic System

GERD

Gastroesophageal Reflux Disease.

- Proton Pump Inhibitors and Acid Pump Antagonists

GH

- Growth Hormone

Ghrelin

A 28 amino acid long peptide hormone, which stimulates appetite and secretion of growth hormone from the anterior pituitary, mainly produced in the stomach. The active form of ghrelin exists as an acylated peptide with O-n-octanoylation at serine 3 essential for its activity. Ghrelin has been identified as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) ►G-protein-coupled receptor. The receptor is known to be activated by small synthetic molecules, called growth hormone secretagogues, which stimulate the release of growth hormone from the pituitary. GHS-R is mainly expressed in hypothalamic areas of the brain where ghrelin is thought to exert its orexigenic effects. The ghrelin system is presently the focus of an intense ongoing research effort to understand the regulation of body weight homeostasis and energy expenditure in order to find new avenues to treat obesity and related disorders.

- Appetite Control
- Anti-obesity Drugs
- G-protein-coupled Receptor
- Orphan Receptors

Giardia lamblia

Giardia lamblia is a ubiquitous, anaerobic flagellate that is responsible for acute and chronic diarrhoea. The cysts of *Giardia lamblia* are passed out with stool and are then ingested orally. In the small intestine they develop into trophozoites which attach to the mucosa. Giardiasis goes along with steatorrhea and, if the infection persists, can cause typical symptoms of malabsorption.

► Antiprotozoal Drugs

GIP

Gastric inhibitory peptide.

► Incretin Hormones

GIRK

GIRK stands for G protein-regulated inwardly rectifying K⁺ channel. GIRK channels are a family of proteins characterized by two membrane spanning domains and a pore-forming domain. GIRK channels are important components of signal transduction pathways regulated by ► [G-protein-coupled receptors](#). GIRK activation results in hyperpolarization and inhibition of neurotransmitter release.

- K⁺ Channels
- Inward Rectifier K⁺ Channels
- Galanin Receptors
- Muscarinic Receptors

Gitelman's Syndrome

A milder clinical course with symptoms usually not apparent before age 5–10, and hypocalciuria with hypomagnesemia has been the clinical distinction between Gitelman's syndrome and true ► [Bartter's](#)

[syndrome](#). The identification of a linkage between NaCl cotransporter (NCC) mutations and the disease provides a justification for this classification, and an explanation for the distinctly different phenotypes.

► Diuretics

Glial Cells

Glial cells are cells within the central or peripheral nervous system which are not immediately involved in information processing. Glial cells play an important role in the metabolic homeostasis of brain tissue and in nervous system development.

Glibenclamide

Sulfonylurea derivative which lowers blood glucose by stimulation of insulin secretion. Enhances the effect of submaximal glucose concentrations on the ATP-dependent potassium channel in pancreatic β -cells. Its potency is 200-fold higher than that of earlier derivatives, and it was therefore designated 2nd generation sulfonylurea. Because of its high affinity to other proteins, glibenclamide (US name: glyburide) is retained in tissues and may cause protracted hypoglycemia in patients who eat irregularly. Extrapankreatic, insulin-like effects of sulfonylurea derivatives have been described in vitro, but require concentrations that largely exceed the therapeutic levels.

► Diabetes Mellitus

Glomerular Filtration Rate

Glomerular filtration rate (GFR) is the volume of plasma-like fluid that is filtered per unit time across the glomerular capillary membranes to enter the tubular space. Filtrate formation is driven by the net filtration pressure that is equal to the capillary hydrostatic pressure diminished by the sum of capillary oncotic

pressure and tubular hydrostatic pressure. Although normally only 1% of the filtered NaCl and water is excreted as urine, reductions in GFR are often accompanied by parallel reductions in urine excretion.

- ▶ Diuretics
- ▶ Pharmacokinetics

GLP-1

- ▶ Glucagon-like Peptide-1
- ▶ Incretin Hormones

Glucagon

Glucagon is a single chain 21 amino acid polypeptide, which is synthesized mainly in the A-cells of the pancreatic islets as well as in the stomach. Glucagon secretion is stimulated by low, and inhibited by high, concentrations of glucose or fatty acids in the plasma. Sympathetic nerves and circulating adrenaline stimulate glucagon release via γ -adrenoceptors. Somatostatin, released from D-cells of the pancreas to the glucagon-secreting A-cells in the periphery of the islets, inhibits glucagon release. One of the main physiological stimuli for glucagon secretion is the concentration of amino acids, in particular arginine, in the plasma. Glucagon acts on the liver to stimulate glycogen breakdown and gluconeogenesis as well as to inhibit glycogen synthesis and glucose oxidation. The net effect is consequently an increase in blood glucose. In liver and fat cells, glucagon induces lipolysis, while in muscle it leads to catabolism of proteins. The actions of glucagon on its major target tissues are thus the opposite of those of insulin. Glucagon exerts its effects through specific receptors, which belong to the group of ▶**G-protein-coupled receptors**. The glucagon receptor is coupled to adenylyl cyclase via the G-protein G_s in a stimulatory fashion. Glucagon can be used clinically to treat hypoglycaemia in unconscious patients under emergency conditions.

- ▶ Gluconeogenesis
- ▶ Insulin Receptor

Glucagon-like Peptide-1 (GLP-1)

Glucagon, glucagon-like peptide-1 (GLP1), and GLP2 are three peptide hormones generated by cleavage of a common precursor polypeptide (preproglucagon). GLP1 stimulates insulin secretion from pancreatic B cells and inhibits feeding in fasted rats when injected into cerebral ventricles.

- ▶ Incretin Hormones
- ▶ Appetite Control
- ▶ Insulin Receptor

Glucocorticoid Receptor

- ▶ Gluco-mineralocorticoid Receptors

Glucocorticoid Resistance

Syndrome characterized by elevated plasma cortisol concentrations, a normal circadian pattern set at a higher level and resistance to adrenal suppression by dexamethasone in the absence of clinical signs of Cushing's syndrome. Although mutations in the glucocorticoid receptor gene have been identified, the etiology of glucocorticoid resistance is not yet fully understood. Acquired forms of glucocorticoid resistance can be observed after long-term hormone therapy, e.g., in asthma or leukemia patients.

- ▶ Gluco-mineralocorticoid Receptors

Glucocorticoids

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Synonyms

Corticosteroids; Glucocorticoid agonists; Steroids; Asthma controllers

Definition

Endogenous glucocorticoids (cortisol) are released from the zona fasciculata of the ►adrenal gland in response to stress. When in excess glucocorticoids can cause ►catabolism of muscle and release of amino acids; these are subsequently used to increase glucose synthesis by the liver (gluconeogenesis). The most important function of glucocorticoids in disease is to regulate the inflammatory response to exogenous stimuli. Exogenous glucocorticoids have been used clinically for over 50 years and have proved to be indispensable in the regulation of a variety of inflammatory and immune states.

Mechanism of Action

Inflammation is a central feature of many chronic diseases including ►bronchial asthma. The specific characteristics of the inflammatory response and the site of inflammation differ between diseases, but all involve the recruitment and activation of infiltrating inflammatory cells and, in asthma, activation of structural cells within the airway is also seen. These diseases are characterised by an increased expression of many mediators including cytokines, chemokines, growth factors, enzymes, receptors and adhesion molecules. Increased inflammatory gene transcription is regulated by pro-inflammatory transcription factors, such as nuclear factor- κ B (►NF- κ B). For example, NF- κ B is markedly activated in epithelial cells of asthmatic patients and this transcription factor regulates many of the inflammatory genes that are abnormally expressed in ►bronchial asthma.

NF- κ B is activated by all the stimuli thought to be important in the inflammatory response to allergen exposure seen in asthma and is the major target for glucocorticoids. NF- κ B is ubiquitously expressed within cells and is able to not only control the induction of inflammatory genes in its own right but can enhance the activity of other cell- and signal-specific transcription factors such as CAAT/enhancer binding protein (C/EBP) α and interferon response factor (IRF)-1. NF- κ B is activated by numerous extracellular stimuli including cytokines, such as tumour necrosis factor- α (TNF α) and interleukin (IL)-1 β , rhinovirus infection and allergen exposure. NF- κ B is held within the cytoplasm in an inactive state by an inhibitor protein I- κ B α . Phosphorylation of I κ B α following cell stimulation leads to the dissociation of I κ B α from NF- κ B, enabling nuclear translocation and binding to specific DNA response elements within the *regulatory regions* of responsive genes.

Due to the large amount of DNA present within the nucleus it must be carefully packaged. In the resting cell DNA is tightly compacted around basic ►histone proteins, excluding the binding of the enzyme ►RNA polymerase II, which activates the formation of ►mRNA. This conformation of the chromatin structure

is described as “closed” and is associated with suppression of gene expression. Expression of genes is associated with enzymatic modification of core histones leading to alterations in chromatin structure. DNA-bound transcription factors recruit ►transcriptional coactivator complexes that contain intrinsic histone acetyltransferase (HAT) activity. This results in acetylation of specific lysine residues locally within the N-terminal histone tail. Acetylation of the σ -group on lysines provides a “mark” or “tag”, which is recognised by other coactivators and by chromatin remodelling engines leading to a relaxed DNA structure and allowing the recruitment of large protein complexes including RNA polymerase II. Not all NF- κ B sites are the same and the degree of NF- κ B activation induced by various stimuli and the activation of additional transcription factors or coactivators results in different patterns of inflammatory genes being up-regulated depending upon the precise cell and stimulus used.

Repression of genes is associated with reversal of this process under the control of histone deacetylases (HDACs). Deacetylation of histones increases the winding of DNA round histone residues, resulting in a dense chromatin structure and reduced access of transcription factors to their binding sites, thereby leading to repressed transcription of inflammatory genes.

Cytokines such as TNF α and IL-1 β , acting via NF- κ B, can induce histone acetylation in both a time- and concentration-dependent manner. Upon DNA binding, NF- κ B recruits transcriptional coactivators such as CREB binding protein (CBP) and p300/CBP-associated factor (PCAF).

Glucocorticoids exert their effects by binding to a cytoplasmic receptor (GR). GRs are expressed in almost all cell types. The inactive GR is bound to a protein complex that includes two subunits of the Heat Shock Protein (►HSP) 90, which thus act as cytoplasmic inhibitors preventing the nuclear localisation of unoccupied GR. Once the ligand binds to GR, HSP90 dissociates allowing the nuclear localisation of the activated GR-steroid complex and its binding as a homodimer to specific DNA sequences (GRES, GGTACAnnnTGTTCT) and interaction with ►coactivator complexes.

Glucocorticoids produce their effect on responsive cells by stimulating GR to directly or indirectly regulate the transcription of target genes. The number of genes per cell *directly* regulated by glucocorticoids is estimated to be between 10 and 100, but many genes are indirectly regulated through an interaction with other transcription factors and coactivator complexes. Glucocorticoids may suppress inflammation by increasing the synthesis of anti-inflammatory proteins, such as annexin-1, IL-10 and the inhibitor of NF- κ B, I κ B- α (Table 1). In addition, it is likely that glucocorticoid side effects, such as ►osteoporosis, cataracts, skin fragility

Glucocorticoids. Table 1 Glucocorticoid-sensitive genes

Increased transcription	Decreased transcription
Lipocortin-1/Annexin-1 (phospholipase A ₂ inhibitor)	Cytokines (IL-1, 2, 3, 4, 5, 6, 9, 11, 12, 13, 16, 17, 18, TNF α , GM-CSF, SCF)
β -Adrenoceptor	Chemokines (IL-8, RANTES, MIP-1 α , MCP-1, MCP-3, MCP-4, eotaxin)
Secretory leukocyte inhibitory protein (SLPI)	Inducible nitric oxide synthase (iNOS)
Clara cell protein (CC10, phospholipase A ₂ inhibitor)	Inducible cyclooxygenase (COX-2)
IL-1 receptor antagonist	Cytoplasmic phospholipase A ₂ (cPLA ₂)
IL-1R2 (decoy receptor)	Endothelin-1
I κ B α (inhibitor of NF- κ B)	NK ₁ -receptors, NK ₂ -receptors
CD163 (Scavenger receptor)	Adhesion molecules (ICAM-1, E-selectin)

and Hypothalamic-Preoptic-Adrenal axis suppression are due to gene activation.

GRs, as with NF- κ B and other transcription factors, increase gene transcription through an action on chromatin modifications and recruitment of RNA polymerase II to the site of local DNA unwinding. GR interacts with CBP and other coactivator proteins, including CBP, PCAF and steroid receptor coactivator-1 (SRC-1), which enhance local HAT activity. This raises the question “how can GR, or any other transcription factor, interact with its recognition site when DNA is compacted”? GR may bind to a GRE within the linker DNA between nucleosomes or alternatively GR may bind to a GRE when the residues which form the GRE are facing outwards from the compacted nucleosomal structure. Binding to the GRE may then modify the local chromatin structure further enabling GR access.

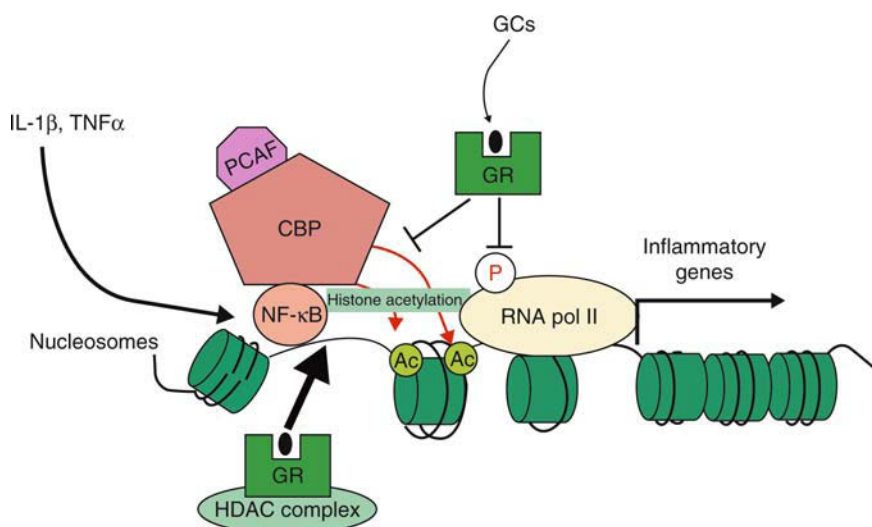
There is now clear evidence that GR does not stably associate with DNA but rather it has a “hit-and-run” mechanism of action. After activation GR tracks along DNA until it finds a GRE and resides on this site for less than 10s before being ejected and replaced by another GR. This ejection may allow binding of additional regulatory factors that enhance gene transcription such as HAT-containing complexes and may also play a role in feedback regulation and subsequent ►**proteosomal degradation** of GR.

In spite of the ability of glucocorticoids to induce gene transcription, the major anti-inflammatory effects of glucocorticoids are through repression of inflammatory and immune genes. The inhibitory effect of glucocorticoids appears to be due largely to an interaction between activated GR and transcription factors, such as NF- κ B and activator protein-1 (AP-1, a heterodimer of Fos and Jun proteins), which mediate the expression of most inflammatory genes. The interplay between pro-inflammatory transcription factors and GR may reflect differing actions on specific NF- κ B/transcription factor/coactivator complexes activated by

differing stimuli resulting in local changes in histone modifications, recruitment of RNA polymerase II and changes in distinct profiles of inflammatory genes. Thus, glucocorticoids are able to attenuate the NF- κ B-mediated induction of histone acetylation by IL-1 β in lung epithelial cells and macrophages. This occurs by a combination of a direct inhibition of NF- κ B-associated HAT activity and by active recruitment of corepressor complexes containing HDAC proteins (Fig. 1). Overall, this results in the deacetylation of histones, increased tightening of DNA round histone residues, loss of RNA polymerase II and repression of inflammatory genes.

►**Mitogen-activated protein kinases** (MAPK) play an important role in inflammatory gene expression through the regulation of pro-inflammatory transcription factors and there is increasing evidence that glucocorticoids may exert an inhibitory effect on these pathways. Glucocorticoids reduce the stability of mRNA for inflammatory genes such as cyclooxygenase-2 (►**COX-2**) through an inhibitory effect on p38 MAP kinase, through rapid induction of a specific p38 MAPK phosphatase (MKP-1) and subsequent dephosphorylation of phospho-p38 MAPK. GR has also been shown to prevent serine phosphorylation of c-Jun and, subsequently, AP-1 activation, by blocking the induction of the Jun N-terminal kinase (JNK) signalling cascade. Consistent with this, glucocorticoids also antagonise other JNK-activated transcription factors such as ETS-Like Kinase 1 (Elk-1) and Activating Transcription Factor 2 (ATF-2). Conversely, JNK can phosphorylate GR and thereby attenuate glucocorticoid responsiveness.

The importance of cross-talk in GR actions is indicated by the construction of a GR dimerisation-deficient mutant mouse in which GR is unable to dimerise and therefore bind to DNA, thus separating the DNA-binding (transactivation) and inflammatory gene repression (transrepression) activities of glucocorticoids. In these animals dexamethasone was able to inhibit AP-1- and NF- κ B-mediated gene transcription,



Glucocorticoids. Figure 1 How glucocorticoids switch off inflammatory genes. Inflammatory genes are activated by inflammatory stimuli, such as interleukin (IL)-1 β or tumor necrosis factor (TNF) α , resulting in activation of the transcription factor nuclear factor κ B (NF- κ B). NF- κ B translocates to the nucleus and binds to specific κ B recognition sites and also to *transcriptional coactivators*, such as CREB-binding protein (CBP) or p300/CBP-activating factor (PCAF), which have intrinsic *histone acetyltransferase* (HAT) activity. This results in acetylation of lysines in *histone* proteins, resulting in recruitment and phosphorylation of *RNA polymerase II* and subsequent increased expression of genes encoding inflammatory proteins. Glucocorticoid receptors (GR), after activation by glucocorticoids (GCs), translocate to the nucleus and, as a monomer, bind to *corepressor* proteins thereby inhibiting NF- κ B-associated HAT activity. In addition, GR is able to recruit corepressor proteins including *histone deacetylases* (HDAC) to the NF- κ B complex further enabling suppression of inflammatory genes. GR may also affect the phosphorylation of *RNA polymerase II* to block inflammatory gene expression.

but the ability to facilitate GRE-mediated effects such as cortisol suppression and T-cell apoptosis were markedly attenuated. This suggests that the development of glucocorticoids with a greater therapeutic window is possible.

Clinical Use (Including Side Effects)

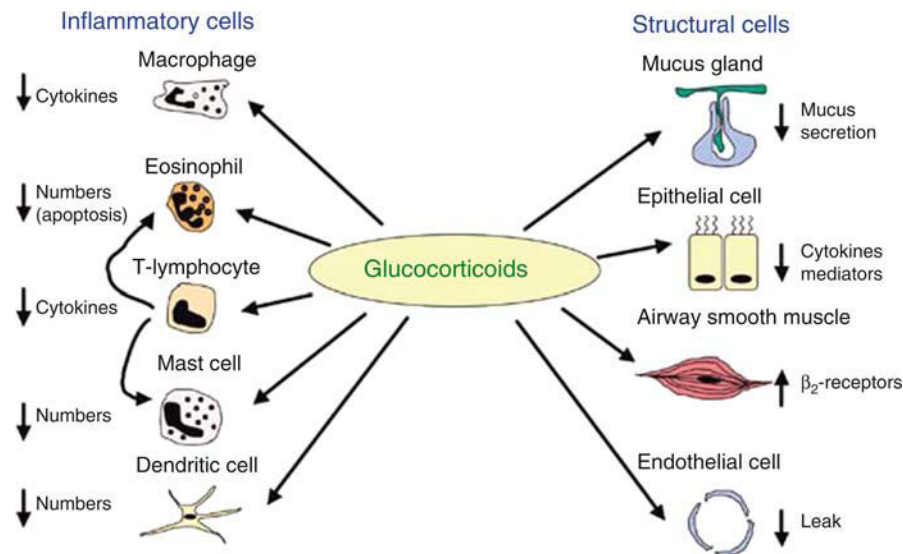
Glucocorticoids are widely used to treat a variety of inflammatory and immune diseases. With the recognition that airway inflammation is present even in patients with mild asthma, treatment with glucocorticoids is now the mainstay of asthma therapy. Consequently, by far the most common use of glucocorticoids today is in the treatment of asthma and inhaled glucocorticoids have now become established as first-line treatment in adults and children with persistent asthma, the commonest chronic airway inflammatory disease.

Inhaled glucocorticoids reduce the number of infiltrating mast cells, macrophages, T-lymphocytes and eosinophils in the airway. Furthermore, glucocorticoids reverse the shedding of epithelial cells and the goblet-cell $\blacktriangleright$ hyperplasia characteristically seen in asthmatic patients (Fig. 2). By reducing airway inflammation, inhaled glucocorticoids reduce $\blacktriangleright$ airway hyperresponsiveness in adults and children with asthma. Current asthma guidelines suggest that the addition of a long-acting β_2 -agonist should be considered rather than

increase the glucocorticoid dose. The rationale for this is that long-acting β_2 -agonists can enhance glucocorticoid receptor activation and inflammatory gene suppression without affecting the side-effect profile.

Although glucocorticoids are highly effective in the control of asthma and other chronic inflammatory or immune diseases, a small proportion of patients with asthma fail to respond even to high doses of oral glucocorticoids. Resistance to the therapeutic effects of glucocorticoids is also recognised in other inflammatory and immune diseases, including rheumatoid arthritis and inflammatory bowel disease. Glucocorticoid-resistant patients, although uncommon, present considerable management problems. It is likely that there is a spectrum of glucocorticoid responsiveness, with the rare resistance at one end, but a relative resistance is seen in patients who require high doses of inhaled and oral glucocorticoids (glucocorticoid-dependent asthma). At present the limiting factor to treating these patients with ever increasing doses of glucocorticoids is the side-effect profile.

Generally, inhaled glucocorticoids have few side effects, the appearance of which depends on the dose, the frequency of administration, and the delivery system used. The most common side effect is dysphonia (hoarseness), which affects approximately one third of treated patients. Oropharyngeal candidiasis (thrush)



Glucocorticoids. Figure 2 Cellular effect of glucocorticoids. Glucocorticoids can affect the activation of most resident and infiltrating cells with the airway suppressing either cell number or mediator release or both. In addition, glucocorticoids are able to decrease vascular permeability (leak) within the airways that causes oedema and increase the expression of β_2 -receptors in smooth muscle cells.

may also be a problem for some patients, particularly the elderly, when the drug is given more than twice daily. There has been some concern that inhaled glucocorticoids may cause stunting of growth in children but recent evidence suggests that this is not a problem even in children treated with higher doses of inhaled glucocorticoids for a long period. Thinning of the skin, telangiectasia, and easy bruising are also classic side effects of both oral and topical glucocorticoids. The easy bruising linked to inhaled glucocorticoids is more frequently seen in elderly patients.

Oral glucocorticoids used to treat severe asthmatic subjects, however, give rise to more serious side effects. Glucocorticoids suppress the hypothalamic-pituitary-adrenal axis by reducing cortisol secretion by the adrenal glands. The degree of suppression depends upon the dose, duration, frequency, and timing of glucocorticoid administration. Oral glucocorticoid therapy also causes osteoporosis with an increased risk of vertebral and rib fractures, but there are no reports suggesting that long-term treatment with inhaled glucocorticoids is associated with an increased risk of fractures. In addition, long-term treatment with oral glucocorticoids increases the risk of posterior subcapsular cataracts; this may be a problem in a few patients taking inhaled glucocorticoids.

Due to the side-effect problems seen with high doses of inhaled glucocorticoids resulting from systemic absorption and the use of oral glucocorticoids in severely affected patients, there has been a search for safer glucocorticoids for inhalation and even for oral administration. As discussed above, a major mechanism

of the anti-inflammatory effect of glucocorticoids appears to be due to the inhibition of the effects of pro-inflammatory transcription factors (*transrepression*). By contrast, the endocrine and metabolic effects of glucocorticoids that are responsible for the systemic side effects of glucocorticoids are likely to be mediated predominantly via *transactivation*. This has led to a search for novel glucocorticoids that selectively *transrepress* without significant *transactivation*, thus reducing the potential risk of systemic side effects.

Several steroidal and non-steroidal glucocorticoid receptor selective dissociated agonists are in development by many pharmaceutical companies and some are now in clinical development. This suggests that the development of dissociated glucocorticoids with a greater margin of safety is possible and may even lead to the development of oral compounds that do not have significant adverse effects.

Now that the molecular mechanisms of glucocorticoids have been elucidated, this raises the possibility that novel non-steroidal anti-inflammatory treatments might be developed, which mimic the actions of glucocorticoids on inflammatory gene regulation. Inhibition of specific HATs activated by NF- κ B may prove useful targets, especially if they also repress the action of other pro-inflammatory transcription factors. Many of the anti-inflammatory effects of glucocorticoids appear to be mediated via inhibition of the transcriptional effects of NF- κ B and small molecule inhibitors of I κ B kinase-2 (IKK2), which activate NF- κ B, are now in development. However, glucocorticoids have additional effects so that it is not certain

whether IKK2 inhibitors will parallel the clinical effectiveness of glucocorticoids and they may have side effects, such as increased susceptibility to infections. Other treatments that have therapeutic potential as glucocorticoid-sparing agents include p38 MAP kinase inhibitors.

- Gluco-mineralocorticoid Receptors
- Immunosuppressive Agents
- Inflammation

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Glucocorticoids, Inhalable

Inhalable glucocorticoids are derivatives of the hormone cortisol, which are applied topically in the treatment of (allergic) ► **bronchial asthma**. In contrast to systemically applied glucocorticoids, these drugs are metabolized upon first liver passage into inactive forms, thus reducing the systemic side effects of unintentionally swallowed drug. Like all glucocorticoids they exert strong anti-inflammatory and anti-allergic effects.

- Glucocorticoids
- Immunosuppressive Agents
- Bronchial Asthma
- Allergy

Glucocorticosteroids

- Glucocorticoids

Gluco-mineralocorticoid Receptors

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Synonyms

Glucocorticoid Receptor: GR; GCR; GRL; Nuclear Receptor Subfamily 3, Group C, Member 1 (NR 3C1); Glucocorticoid Receptor Type II

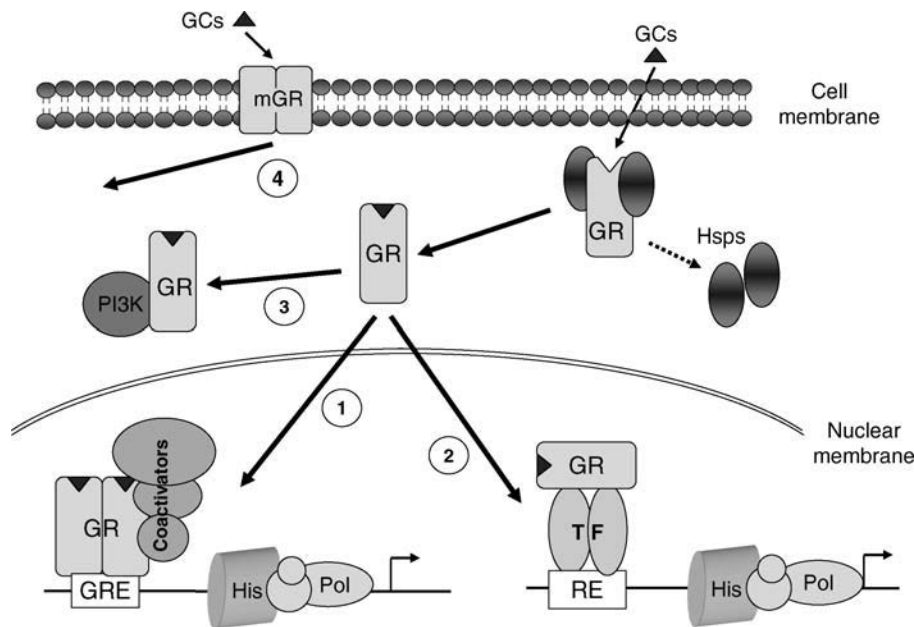
Mineralocorticoid Receptor: MR; MCR; MRL; Nuclear Receptor Subfamily 3, Group C, Member 2 (NR3C2); Glucocorticoid Receptor Type I; Aldosterone Receptor

Definition

Glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) are members of the ► **nuclear receptor superfamily** and they mediate the organism's response to cortisol and aldosterone, two steroid hormones synthesized in the adrenal gland [1]. GR is ubiquitously expressed and orchestrates a plethora of physiological processes including energy homeostasis, stress response, and inflammation. In contrast, expression of MR is largely restricted to epithelial cells in kidney and colon as well as the limbic system of the brain. Consequently, MR mainly controls water homeostasis and cognitive processes. Due to their central roles in many physiological processes, lack of either of the two receptors is incompatible with life.

Basic Characteristics

In the inactive state, both GR and MR reside in the cytoplasm bound to a complex of heat shock proteins (Hsps). The main ligands cortisol and aldosterone can freely cross the cell membrane owing to their lipophilic nature. Upon hormone-binding, the receptor is released and translocates into the nucleus (Fig. 1). Following homodimerization, GR and MR bind to specific response elements located in the promoter and enhancer region of responsive genes and thereby induce gene expression. Thus, both GR and MR act as transcription factors (TFs). In addition, evidence has accumulated that the two receptors may also heterodimerize, resulting in synergy or inhibition of transcription. Besides these DNA-binding-dependent mechanisms, GR only interacts with other transcription factors without direct DNA-contact itself [1]. This second mode of GR action, also known as ► **cross talk**, plays an important role in the regulation of the immune system. Furthermore, so-called nongenomic effects, occurring within seconds or minutes after hormone



Glucocorticoid Receptors. Figure 1 Molecular modes of glucocorticoid receptor action. Glucocorticoids (GCs) passively enter the cell and bind to GR, resulting in the release of associated heat shock proteins (Hsps). After translocation into the nucleus, GR modulates transcription either by binding of homodimers to glucocorticoid response elements (GREs) (1) or via protein–protein interaction with other transcription factors (TFs) (2). Alternatively, GRs interact with cytosolic signaling molecules such as PI3K (3). Finally, glucocorticoids presumably bind to yet uncharacterized membrane GRs (mGRs) and thereby elicit fast responses (4). His = histones, Pol = RNA Polymerase II.

exposure, have been described for both GR and MR. These fast responses may either be mediated by interaction with cytosolic signaling molecules such as PI3K or by binding of hormones to membrane-bound receptors. However, whether the latter ones are encoded by the classical GR and MR genes or rather represent unrelated proteins remains unknown.

Molecular Characterization of Glucocorticoid and Mineralocorticoid Receptor

The cDNA of GR was first cloned in 1985 [2]. GR is a 94 kDa protein and consists of 777 (alpha) amino acids in the human. The cDNA of MR was cloned 2 years later by homology screening and it represents a 107 kDa protein consisting of 984 amino acids in the human. GR and MR are formed of a modular structure consisting of three major domains, common to most members of the nuclear receptor superfamily. The central DNA-binding domain is the most conserved one and it consists of two Zinc-fingers. The first one is responsible for DNA-binding whereas the second one contributes to dimerization of the receptor. The C-terminal region is made of the ligand-binding domain, which is responsible for hormone binding and interpretation. Additionally, the hormone-dependent transactivation domain AF-2 and a nuclear-localization signal are located here. The

N-terminal domain is the least conserved region and contains the transactivation domain AF-1.

Human glucocorticoid receptor maps to the long arm of chromosome 5 (5q31–5q32) whereas human mineralocorticoid receptor maps to the long arm of chromosome 4 (4q31.1).

Between 5,000 and 100,000 GR molecules are found within almost every cell of the organism. The affinity constant for cortisol comes to around 30 nM, which is in the range of the concentration of free hormone in the plasma under normal conditions. Consequently, receptor occupancy can be expected to be 10–70%. This suggests that changes in cortisol secretion such as under stress conditions, directly translate into alterations in GR occupancy, leading to transcriptional responses.

Although MR also binds glucocorticoids, its main ligand in classical mineralocorticoid target tissues such as kidney and colon is aldosterone (K_d 1.3 nM). This can be granted to the ability of [11 \$\beta\$ -hydroxysteroid dehydrogenase type II](#) (11 β -HSD II) to convert active cortisol into its inactive metabolite cortisone in these tissues. Since aldosterone is no substrate for this enzyme it can readily bind to MR, leading to exclusive occupation of the receptor by aldosterone. In contrast, no such mechanism exists in brain and presumably

heart, resulting in predominant glucocorticoid-binding to MR. Since MR has an approximately tenfold higher affinity for cortisol than GR, it is almost completely occupied by hormone already under basal conditions.

Control of Glucocorticoid Secretion by the Hypothalamus–Pituitary–Adrenal Axis

Synthesis and secretion of glucocorticoids is controlled by a neuroendocrine cascade called the ►**hypothalamus pituitary adrenal (HPA) axis**. Stimuli such as stress or infection lead to the release of corticotropin-releasing hormone (CRH) in the hypothalamus. CRH is transported to the anterior pituitary, where it induces increased synthesis and secretion of adrenocorticotrope hormone (ACTH). Finally, ACTH stimulates release and production of glucocorticoids in the adrenal cortex. Protection from chronically elevated hormone levels is achieved by a negative feedback-loop where glucocorticoids inhibit their own production and secretion by binding to GR, mainly in the pituitary and in the hypothalamus. Further regulation comprises effects of GR and MR on glucocorticoid production exerted via higher brain centers such as the hippocampus. Superimposed to the regulation by the HPA axis, glucocorticoid serum levels show a circadian rhythm with high levels in the morning and low levels in the evening (in the human). Finally, a feedback loop connecting glucocorticoid production with the control of the immune system is operative. Cytokines released during inflammation stimulate glucocorticoid secretion, which in turn inhibits cytokine production. This shows that glucocorticoids acting via GR and MR are under complex regulation by the endocrine, immune, and central nervous system.

Physiological Functions of Glucocorticoid Receptor

Glucocorticoids derive their name from the finding that they play an important role in carbohydrate metabolism. They increase hepatic glycogen and gluconeogenesis, decrease glucose uptake and utilization in peripheral tissues and cause a tendency to hyperglycemia and reduced glucose tolerance. Furthermore, energy homeostasis is also influenced via effects on protein and lipid metabolism. An important function of glucocorticoids, which are widely employed in the treatment of autoimmune diseases, is their potent anti-inflammatory and immunosuppressive activity. Glucocorticoids inhibit the production and release of cytokines (IL-1 β , IL-6, TNF α), chemokines (IL-8), and various enzymes (COX-2, iNOS) and induce T cell apoptosis. Furthermore, they interfere with proliferation, leukocyte migration, and the generation of adaptive immune responses. In the central nervous system, glucocorticoids contribute to mood and memory formation. Further processes that are controlled by GR include erythropoiesis, bone formation, adrenalin

synthesis, and lung function. This shows that a plethora of physiological processes involves regulation by GR, also explaining the variety of symptoms observed in diseases related to GR dysfunction or after genetic manipulation in animals (see below).

Diseases Related to Glucocorticoid Dysregulation

The most common disease related to glucocorticoids is Cushing's syndrome. This disorder is characterized by hypercortisolism due to a pituitary or adrenal tumor. The elevated glucocorticoid levels lead to a large spectrum of symptoms, including obesity, hirsutism, hypertension, muscular weakness, depression, osteoporosis, short stature, facial plethora, fat redistribution, and atrophy of the skin. Treatment of Cushing's disease is either achieved by surgical removal of the tumor or by pharmacological interference with ACTH or cortisol hypersecretion. Addison's disease, in contrast, is caused by adrenocortical insufficiency mainly following autoimmune destruction or tuberculosis. Clinical features of hypocortisolism include fatigue, weight loss, anorexia, and hypotension. Treatment can only be achieved by life-long replacement therapy with both glucocorticoid and mineralocorticoid hormones. In contrast to the aforementioned syndromes, congenital adrenal hyperplasia (CAH) has a genetic cause. Mutations in genes encoding steroidogenic enzymes impair the production of both glucocorticoid and mineralocorticoid hormones. As a consequence of the disturbed negative feedback mechanism, ACTH levels are elevated resulting in enhanced androgen production by the adrenal gland. Finally, depression is also linked to GR and MR function in a yet not fully understood way. Elevated glucocorticoid levels and a blunted circadian rhythm usually accompany depressive illnesses, which both normalize after application of anti-depressants. However, whether glucocorticoids are cause or effect of depression remains to be elucidated.

Clinical Features and Genetic Basis of Glucocorticoid Resistance

Primary ►**glucocorticoid resistance** in humans is characterized by elevated plasma cortisol concentrations, a normal circadian pattern set at a higher level and resistance to adrenal suppression by dexamethasone in the absence of clinical signs of Cushing's syndrome. The absence of hypercortisolism is presumably due to the fact that glucocorticoid resistance is in balance with peripheral tissues, being as resistant to hormone as the central tissues of the HPA axis. In severe forms, primary glucocorticoid resistance is accompanied by an increase in mineralocorticoid secretion leading to hypertension and hypokalemic alkalosis. In women, overproduction of androgens causes signs of hirsutism. The causes of glucocorticoid insensitivity are diverse and not yet fully understood. For example, point mutations in

the GR gene leading to reduced hormone binding or transcriptional capacity, reduced number of receptor molecules due to heterozygosity, or splice-site mutations and changes in receptor associated proteins all have been accounted for the observed clinical symptoms. Additionally, resistance to glucocorticoids may also be acquired, following long-term glucocorticoid treatment of leukemia or asthma patients.

Animal Models for Glucocorticoid Receptor Function

Pepin et al. (1992) generated transgenic mice in which antisense RNA complementary to GR cDNA led to reduced expression mostly in neuronal tissues. Consequently, this was found to result in an impaired behavior, a defective response to stress as well as in obesity. King et al. (1995) generated transgenic mice where reduced GR expression was limited to the thymus. This leads to an altered thymocyte development, changes in the T-cell repertoire, and a reduced risk to develop autoimmune diseases.

Cole et al. (1995) reported on ►**knock-out mice** with a germ line deletion of GR. They demonstrated that lack of GR leads to perinatal death, atelectasis of the lung, and lack of adrenalin synthesis. To circumvent perinatal lethality, Tronche et al. (1999) and Brewer et al. (2003) generated tissue-specific somatic deletions of GR. This allowed to characterize GR function in the CNS, the immune system, and the liver in more detail. In particular, these approaches revealed novel aspects of organ-specific glucocorticoid physiology such as anxiety-like behavior, growth control, and polyclonal T cell activation.

Since GR can influence transcription through both the DNA-binding-dependent and-independent mechanisms, Reichardt et al. [3] attempted to separate these two modes of action by introducing a point mutation into GR. This mutation interferes with transactivation while transcriptional regulation via cross talk with other transcription factors such as AP-1 and NF- κ B remains intact (see above). In contrast to GR-deficient mice, these GR^{dim} mice are viable, revealing the importance of DNA-binding-independent transcriptional regulation by GR in vivo. Mutant mice lose the ability to transactivate gene transcription by cooperative DNA-binding, which results in impaired thymocyte apoptosis, erythropoiesis, gluconeogenesis, and memory formation. In contrast, most immunosuppressive and anti-inflammatory effects of glucocorticoids are functional in the absence of DNA-binding by GR.

To study the effect of an increased gene dosage of GR, Reichardt et al. (2000) and van den Brandt et al. (2007) generated rodent models of GR overexpression. These approaches confirmed that GR controls neuronal and immune functions in a dosage-dependent manner. These results highlight the importance of tight control of GR expression in target tissues and may explain

differences in the susceptibility of humans to inflammatory diseases and stress.

Physiological Functions of Mineralocorticoid Receptor

MR forms an essential component of the ►**renin–angiotensin–aldosterone system** (RAAS), which regulates salt and water homeostasis in the body. In particular, MR mediates the organism's response to aldosterone in tissues such as kidney, colon, and salivary gland. In the distal tubules of the kidney, it induces enhanced sodium and water retention in response to a reduction in extracellular fluid volume or a fall in blood pressure. Besides its role in salt and water homeostasis, MR appears to play a major role in the limbic system of the brain. Notably, high levels of MR are expressed in hippocampus and amygdala, where it modulates the transcriptional response to glucocorticoid hormones in concert with GR. Studies with specific MR ligands as well as analyses of genetically manipulated mice have indicated that MR in the brain is involved in the control of cognitive processes such as learning and memory and in the modulation of mood and anxiety.

Diseases Related to Mineralocorticoid Receptor

Several diseases involving dysregulation of MR function have been described although most of them are not causatively linked to the receptor itself. Pseudohypoaldosteronism for example is a syndrome of mineralocorticoid resistance characterized by urinary salt loss and dehydration. However, only very rarely mutations in the MR gene have been found in these patients so far. In most cases, this syndrome appears to be linked to defects in the subunits of the amiloride-sensitive sodium channel ENaC, a major target of mineralocorticoid action in the kidney.

Patients suffering from hyperaldosteronism usually present with severe hypertension and marked sodium retention, due to high levels of circulating aldosterone. Whereas primary forms of this disease are caused by certain tumors of the adrenal cortex, resulting in increased hormone production (Conn's disease), secondary forms can often be attributed to enhanced secretion of renin. In contrast, patients with apparent mineralocorticoid excess (AME) show unremarkable levels of aldosterone and cortisol. In fact, this syndrome can be explained by recessive mutations in the gene encoding 11 β -HSD II (see above). Diminished or absent activity of the enzyme leads to massive activation of MR in kidney by the comparably high concentrations of circulating cortisol and consequently to strong sodium and water retention.

Animal Models for Mineralocorticoid Receptor Function

Berger et al. (1998) generated MR knock-out mice by gene targeting. Until day 10 after birth, these mice develop normally. Later they show symptoms of pseudohypoaldosteronism, characterized by massive

loss of renal sodium and water, finally leading to death. The lack of MR causes a severe upregulation of the RAAS with a strong increase in most of its components. Using a salt replacement protocol, it was possible to rescue the mutant mice from postnatal death, thus facilitating the study of the physiological role of MR in the adult. Analyses of the brain revealed that loss of MR caused increased neurodegeneration in the hippocampus accompanied by reactive gliosis and decreased neurogenesis. Forebrain-specific MR knock-out mice reported by Berger et al. [4] confirmed that MR is involved in the control of learning and memory but not of anxiety-like behavior. This highlights distinct roles of GR and MR in the regulation of cognitive functions.

Drugs

GR Ligands

Glucocorticoid agonists mainly comprise cortisol, corticosterone, aldosterone, and synthetic steroids used in clinical therapy such as ►[dexamethasone](#), prednisolone, methylprednisolone, or triamcinolone. In addition, also some nonsteroidal compounds such as aziridines may bind to GR and regulate its activity. Agonists are defined as compounds, which bind to the receptor and elicit a transcriptional response. Cortisol and corticosterone have equal affinities for GR. However, due to the concentration of circulating hormone, cortisol is the principal glucocorticoid in humans and corticosterone in rodents. Aldosterone also displays high affinity for GR but since its concentration in plasma is about three magnitudes of order lower than that of cortisol, aldosterone does not play a physiological meaningful role for GR activity.

Glucocorticoid antagonists also bind specifically to GR. However, these steroids do not elicit a response but rather compete with agonists for binding and thereby prevent an agonist response. In addition, partial agonists are known, which elicit an intermediate response of GR. Well-known examples for glucocorticoid antagonists are ►[RU486 \(Mifepristone\)](#), which also binds to progesterone receptor, and the unrelated compound ZK98299, a presumably pure GR ligand. However, RU486 is not a full antagonist since this compound cannot inhibit some transcriptional activities of GR.

Synthetic glucocorticoids may be grouped in several classes. First, compounds that are more potent than naturally occurring glucocorticoids due to a higher binding affinity or decreased clearance rate. Due to both these effects, dexamethasone, for example, is over 10 times more potent than cortisol. Second, compounds which have less mineralocorticoid activity resulting in a reduced spectrum of MR-mediated side effects. Third, dissociating compounds that are unable to induce transcriptional responses which are mediated by DNA-binding of GR but which are still functional in

modulating transcription by interaction with other transcription factors (see above). This characteristic is expected to be useful in achieving potent anti-inflammatory and immunosuppressive effects (thought to be primarily mediated by interaction with other transcription factors) but lacking some of the adverse effects (presumably mediated by DNA-binding-dependent transcriptional regulation by GR). This principle has gained support from an animal model (GR^{dim}, described above), which mimics this dissociating principle in vivo.

MR Ligands

MR has a high affinity for mineralocorticoids such as aldosterone and DOC. In addition, MR also binds glucocorticoids although in mineralocorticoid target tissues this is prevented by the enzyme 11 β -HSD II (see above). Since mineralocorticoids are no substrate for 11 β -HSD II due to their cyclic 11,18-hemiacetyl-group, they are able to bind to MR despite the 1,000-fold lower concentration in plasma as compared with cortisol.

The main mineralocorticoid agonist in humans is aldosterone. Additionally, cortisol, corticosterone, and DOC have also mineralocorticoid agonistic activity. The synthetic steroid fludrocortisone (9 α -fluorocortisol) is extremely potent and usually chosen for replacement mineralocorticoid therapy. In contrast, aldosterone and DOC are not useful in oral therapy due to rapid degradation in liver after absorption.

Mineralocorticoid antagonists include RU26752, spironolactone, eplerone, and progesterone. Whereas endogenous progesterone may only play a role during the third trimester of pregnancy, ►[spironolactone](#), eplerone, and RU26752 are synthetic drugs, which have been developed for the treatment of pathological states of sodium-regulation and hypertension. These drugs bind to MR with equal affinity as aldosterone but are assumed to induce a transcriptionally silent state.

►Nuclear Receptors

►Glucocorticoids

►Aldosterone

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Gluconeogenesis

Synthesis of glucose from glycerol, lactate, and amino acids. This pathway is essential to maintain normal blood glucose during fasting. ► **Glucagon**, the predominant hormone regulating carbohydrate metabolism during fasting, stimulates gluconeogenesis by inducing the expression of two rate-limiting enzymes (phosphoenolpyruvate carboxykinase and glucose-6-phosphatase). Insulin inhibits expression of these enzymes and reduces gluconeogenesis.

► Insulin Receptor

Glucose Transport Facilitators (GLUT)

Definition

A family of related, membrane-spanning glycoproteins that catalyze the transport of glucose across a lipid bilayer of the plasma membrane along a concentration gradient.

► Glucose Transporters

Glucose Transporters

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Synonyms

(GLUT1–14; gene symbols: *SLC2A1–14*, *solute carrier family 2A1–14*); (sodium–glucose symporters (SGLT1–6); gene symbols: *SLC5A1–6*, *solute carrier family 5A1–6*)

Definition

Glucose transporters are integral membrane proteins that catalyze the permeation of sugars into cells, along or against a concentration gradient.

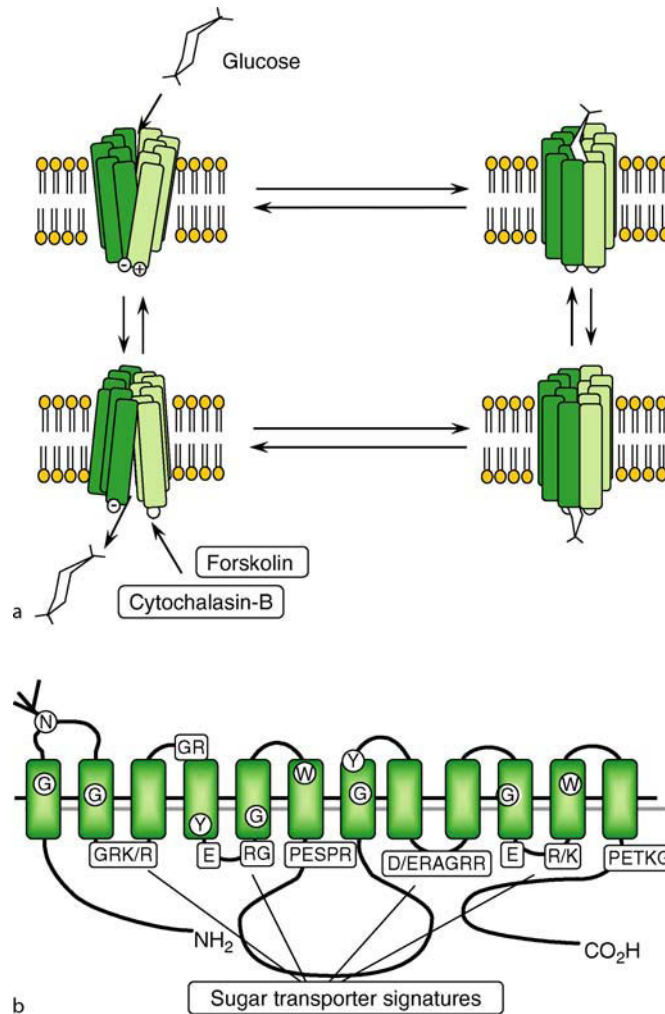
Basic Characteristics

The Family of Glucose Transport Facilitators

Glucose transport facilitators (GLUT proteins) are uniporters which catalyze the diffusion of glucose into (or out of) cells along the concentration gradient [1, 2]. During this process, the proteins are believed to undergo specific conformational changes: Binding of glucose to the outward-facing binding site induces a conformational alteration that moves the substrate through the pore of the GLUT protein. Thereafter, glucose is released from the inward-facing binding site to the cytoplasm, and the transporter undergoes the reverse conformational change (Fig. 1a). Within most cells, glucose is rapidly phosphorylated and metabolized. Thus, under normal conditions the influx of glucose into cells does not alter its concentration gradient. In liver, kidney, and intestinal mucosa, GLUT proteins catalyze the efflux of glucose from cells, when the intracellular glucose concentration exceeds the serum glucose concentration.

The family of GLUT proteins comprises 14 structurally related members, GLUT1–12, HMIT, GLUT14 (29–65% identity). Among these, there are glucose (GLUT1–3, 4, 8, 14), fructose (GLUT5, 7, 11), polyol (GLUT12), and myo-inositol (HMIT) transporters [1, 2]. At present, the function of the other family members is incompletely characterized. The presumed secondary structure of all GLUT proteins is similar, with 12 membrane spanning helices, intracellular N- and C-termini and a large cytoplasmic loop. The three-dimensional structure of a structurally related transporter protein, the glycerol-3-phosphate transporter of *Escherichia coli*, confirmed the proposed model of GLUT proteins and of all other related transporters [2]. GLUT proteins carry charged residues at the intracellular surface of the proteins which are believed to provide the proper orientation and anchoring of the helices in the membrane, and to participate in the conformational changes during the transport process. Several sequence motifs, the sugar transporter signatures, are conserved in all family members, and are essential for the function of the proteins (Fig. 1b).

According to a comparison of the sequences, the GLUT family can be divided into three subclasses [1, 2]. Class I comprises the thoroughly characterized members GLUT1–4 and GLUT14 that are distinguished mainly by their tissue distribution (GLUT1, erythrocytes, brain microvessels; ► **GLUT2**, liver, pancreatic islet – several mutations within the GLUT2 encoding gene *SLC2A2* cause the ► **Fanconi-Bickel syndrome** – ; GLUT3, neuronal cells; ► **GLUT4**, muscle, adipose tissue; GLUT14, testis), their affinity to glucose, and their hormonal regulation. Class II comprises the fructose-specific transporter GLUT5 (testis, intestine, muscle) and three related proteins, GLUT7 (intestine, testis, and prostate), GLUT9



Glucose Transporters. Figure 1 (a) Proposed model of the mechanism of glucose entry into cells by facilitated diffusion. Glucose binds to an outward-facing site of the GLUT protein and induces a conformational change that moves the hexose through a pore in the protein. After glucose is released from its inward-facing binding site, the GLUT protein undergoes the reverse conformational change. Two inhibitory ligands (cytochalasin B and forskolin) bind to the cytoplasmic site of GLUT proteins. (b) Schematic model of the GLUT proteins with their predicted 12 membrane spanning helices. The figure highlights motifs that are considered specific for the GLUT family (sugar transporter signatures), and other residues that are highly conserved in all members of the family.

(pancreas, kidney, liver), and GLUT11 (heart, muscle, pancreas, placenta, kidney). For GLUT7 and GLUT11, fructose-inhibitable glucose transport activity has been demonstrated after expression of their mRNA in *Xenopus* oocytes. Class III comprises five isoforms: GLUT6 (brain, spleen, leukocytes), GLUT8 (testis, brain, adipocytes), GLUT10 (pancreas, liver), GLUT12 (heart, prostate), and HMIT, a myo-inositol transporter (brain). Glucose transport activity has been shown for GLUT6 and GLUT8.

Glucose transport activity is regulated through transcriptional and translational control of the GLUT proteins, through their activity, and through alterations of their intracellular distribution. Most importantly, the GLUT4 continuously cycles between an intracellular,

vesicular storage compartment and the plasma membrane of adipose and muscle cells [2, 3]. In basal cells, most transporters are sequestered in the intracellular compartment. In the presence of insulin, the translocation of GLUT4-containing vesicles to the plasma membrane is markedly accelerated, resulting in an increase in glucose transport uptake (see chapter Diabetes Mellitus). Proteins that presumably participate in this process are ►VAMP2, ►SNARE proteins, ►syntaxin 4, ►SNAP23, and Synip. In some cells, GLUT1 may also be sequestered through a discrete vesicular pathway, and translocated to the plasma membrane in response to energy depletion or lack of glucose.

For some of the GLUT isoforms, the phenotype of null mutants is known. Haploinsufficiency of the GLUT1 in

humans causes a syndrome of low glucose levels in the cerebrospinal fluid and drug-resistant seizures. Disruption of the *GLUT2* gene in mice leads to impaired insulin secretion and diabetes mellitus. In contrast, hepatic glucose output is normal in GLUT2-null mice, suggesting that an alternative pathway for glucose export from hepatocytes exists. Muscle-specific deletion of the GLUT4 results in a reduction of basal glucose transport and a near-absence of the effect of insulin, leading to hyperinsulinemia and impaired glucose tolerance. Furthermore, it is believed that impaired GLUT4 translocation in skeletal muscle is an important factor in the pathogenesis of insulin resistance in obesity and type 2 diabetes. In animal models of morbid obesity, transgenic overexpression of GLUT4 results in increased glucose uptake in muscle, and in improved whole body glucose disposal. Thus, strategies designed to enhance expression and/or translocation of GLUT4 might lead to an effective treatment of insulin resistance and type 2 diabetes [3]. Recently, it was demonstrated that deletion of GLUT3 causes early pregnancy loss during neurulation; the heterozygous mice showed fetal growth retardation but survived birth and developed normal body weight, body composition, glucose, and insulin tolerance. In contrast, GLUT8 was shown to be dispensable for embryonic development. GLUT8 null mutants had normal postnatal development, glucose homeostasis, and response to stress. They showed increased proliferation of hippocampal cells without defects in memory acquisition and retention [4].

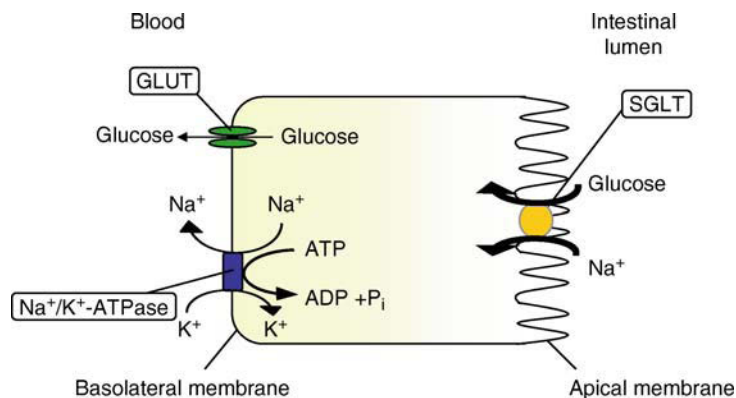
The Family of Sodium-dependent Glucose Cotransporters

Sodium-dependent glucose cotransporters (SGLT) are located on small-intestine and kidney brush-border membranes. SGLT1, SGLT2, and SGLT3 are

structurally different sodium–glucose cotransporters with 59–75% identity, and no homology with the GLUT [5]. They catalyze glucose transport into the cell against a concentration gradient. This transport process is a cotransport of one glucose and of one (for SGLT2) or two (for SGLT1 and SGLT3) Na^+ ions in the same direction. The energetically favored movement of a Na^+ ion through the plasma membrane into the cell, driven both by its concentration gradient and by the membrane potential, is coupled to the movement of the glucose molecule. SGLT proteins exhibit binding sites for glucose and Na^+ on their exofacial surface. The simultaneous binding of Na^+ and glucose to these sites induces a conformational change, generating a transmembrane pore that allows both Na^+ and glucose to pass into the cytosol. After this passage, the proteins revert to their original conformation. In the steady state, Na^+ ions transported from the intestinal lumen into the cells are pumped by a Na^+/K^+ -ATPase across the basolateral membrane (Fig. 2). Glucose concentrated inside the cell by the symport moves outward through the basolateral membrane via GLUT.

The secondary structure of SGLT1 differs from that of other members of the family. As compared with SGLT2 and 3, SGLT1 contains a hydrophobic C-terminus which is assumed to form a 14th transmembrane. SGLT1 is a high-affinity transporter that mediates the sodium–glucose cotransport across the intestinal brush-border membrane. SGLT1 is also expressed in the proximal tubule S3 segments of the kidney. Mutations within the SGLT1 gene (*SLC5A1*; on chromosome 22q12.3) are described in patients suffering from ►[glucose/galactose malabsorption](#).

SGLT2 is a low-affinity, high capacity sodium–glucose cotransporter located in the early proximal convoluted tubule S1 segment. SGLT2 comprises 13



Glucose Transporters. Figure 2 Transport of glucose in intestinal epithelium. Entry of glucose into the epithelial cells is catalyzed by a sodium-dependent cotransporter (SGLT) located in the apical membrane. The Na^+/K^+ ATPase in the basolateral membrane generates the Na^+ gradient that drives the sodium–glucose cotransport by SGLT against the concentration gradient of glucose. Glucose leaves the cell via a facilitated glucose transporter (GLUT) located in the basolateral membrane.

membrane spanning domains. In contrast to SGLT1, SGLT2 does not transport D-galactose. It has been suggested that a defect in the *SGLT2* gene (*SLG5A2* on chromosome 16p11.2) is responsible for ►renal glucosuria.

SGLT3 is also a low-affinity sodium–glucose cotransporter. SGLT3 mRNA was mainly detected in intestine, followed by spleen, liver, kidney, and muscle. SGLT3 comprises of 13 membrane spanning domains. SGLT3 has a lower affinity for Na⁺ than SGLT2 under identical sugar concentrations.

Over recent years, data collected from homology cloning and the Human Genome Project has indicated the presence of additional members of the SGLT-like transporters, which are still under investigation. Additional members (SGLT4–6) have been assigned but await complete functional and structural characterization. Amino acid comparisons of human SGLTs range between 57 and 71% sequence identity; they exhibit no homology with the facilitated glucose transporters [5].

Drugs

Insulin

At present, the only available drug that stimulates glucose transport is insulin. Insulin increases the abundance of the GLUT4 in plasma membranes of adipose and muscle cells by its recruitment from intracellular storage sites (for a detailed description of its mechanism, see Chapter Diabetes Mellitus).

Phlorizin

Phlorizin (phloretin-2'- β -glucoside) is a plant product from the bark of the apple tree which inhibits intestinal glucose absorption and renal reabsorption in proximal tubules by binding to SGLTs. This effect has been used to correct hyperglycemia in experimental, diabetic animals. SGLT2 has a higher affinity for the inhibitor than SGLT1.

Phloretin

Phloretin is the aglycon of phlorizin and inhibits the facilitated diffusion of glucose catalyzed by GLUT1 or GLUT4. It has been used to terminate the uptake of glucose in timed assays with isolated membranes or reconstituted transporters.

Cytochalasin B

Cytochalasin B is a fungal metabolite which binds to the internal site of helices 10 and 11 of the GLUT proteins, and potentially inhibits glucose transport in cells or isolated membrane vesicles ($K_D = 100$ nM). Because cytochalasin B binding can be inhibited by glucose in a competitive manner, it is believed that the ligand binds to the internal glucose binding site of the transporter.

Forskolin

The plant product forskolin (from *Coleus forskolii*) is a diterpene which directly stimulates adenylate cyclase. In addition, the agent potently inhibits glucose transport independent from changes in adenylate cyclase activity. Specific binding of forskolin to GLUT1 or GLUT4 is inhibited by glucose and cytochalasin B in a competitive manner, indicating that the ligand binds to a domain involved in glucose binding. Its binding site has been mapped to helix 8–10 of GLUT1/GLUT4 with the aid of the photoreactive 3-[¹²⁵I]odo-4-azido-phe-nethylamino-7-O-succinyldeacetyl-forskolin (IAPS-forskolin).

►Insulin Receptor

►ATP-dependent K⁺ Channels

►Diabetes Mellitus

►Antidiabetic Drugs other than Insulin

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Glucose/Galactose Malabsorption (GGM)

Glucose/galactose malabsorption (GGM) is an intestinal monosaccharide (glucose and galactose) transport deficiency. The disorder manifests itself within the first weeks of life. The severe diarrhea and dehydration are usually fatal unless glucose and galactose are eliminated from the diet. Fructose and xylose are absorbed normally. Occurrence in both males and females, familial incidence, in particular in parental consanguinity, indicate autosomal recessive inheritance of

GGM. Several missense mutations in human sodium-dependent glucose transporters (SGLT1) have been described that cause GGM.

► Glucose Transporters

α -Glucosidase

► Antidiabetic Drugs Other than Insulin

GLUT

GLUT stands for Glucose Transport Facilitators.

► Glucose Transporters

GLUT2

GLUT2 is a glucose/fructose transport facilitator expressed in liver, small intestine, kidney, and pancreatic β -cells. GLUT2 has low-affinity for glucose ($K_m=60$ mM) and fructose ($K_m=65$ mM), and is an essential part of the glucose sensor of pancreatic β -cells which controls insulin secretion and biosynthesis.

► Glucose Transporters

GLUT4

GLUT4 is a glucose transporter exclusively expressed in tissues with insulin-sensitive glucose uptake (heart, muscle, fat). Under basal conditions, GLUT4 is predominantly located in intracellular vesicles, and is

translocated to the plasma membrane in response to insulin or increased muscular activity. GLUT4 exhibits a high-affinity for glucose ($K_m=4$ mM).

► Glucose Transporters
► Insulin Receptors

Glutamate

Glutamate is a small amino acid which constitutes the most important neurotransmitter at excitatory synapses in the mammalian brain. Glutamate can act on several different types of receptors including cation channels and ►G-protein-coupled receptors.

► Glutamate Receptors
► Ionotropic Glutamate Receptors
► Metabotropic Glutamate Receptors

Glutamate Receptors

Glutamate receptors are classified as AMPA, NMDA, and mGluR. All three subtypes are highly expressed at nociceptive synapses. AMPA and NMDA receptors are agonist-gated cation channels, which depolarize synaptic membranes upon activation. Ion channels associated with AMPA receptors demonstrate fast activation and inactivation kinetics and mediate rapid excitatory neurotransmission. Owing to their slow kinetic properties, their high Ca^{2+} permeability and their blockade by Mg^{2+} under physiological synaptic conditions, NMDA receptors potentiate synapses in several neural pathways, including those involved in chronic pain. mGluRs are ►G-protein-coupled receptors, which couple to G-proteins of the Gq family or the Gi/o family. Their activation at spinal synapses leads to the facilitation of postsynaptic responses and enhanced neurotransmitter release via calcium-mediated activation of intracellular signaling kinases.

► Ionotropic Glutamate Receptors
► Metabotropic Glutamate Receptors
► G-protein-coupled Receptors
► Pain and Nociception
► Analgesics
► Psychotomimetic Drugs

γ -Glutamyl-transpeptidase

This is the enzyme that converts Leukotriene C₄(LTC₄) to Leukotriene D₄ (LTD₄) upon its secretion from inflammatory cells.

► Leukotrienes

Glycine

► Glycine Receptor

Glycine Receptors

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Synonyms

Inhibitory glycine receptor; Strychnine-sensitive glycine receptor; Glycine-gated chloride channel; Glycine-gated anion channel

Definition

The amino acid glycine serves as an important mediator of synaptic inhibition, predominantly in brain stem, spinal cord and retina. Binding of the neurotransmitter to its postsynaptic receptors is antagonized by strychnine, a convulsant alkaloid from *nux vomica* [1]. The inhibitory action of glycine is distinct from its function as a coagonist to glutamate at a strychnine-resistant binding domain of the NMDA receptor. Inhibitory glycine receptors represent a family of ligand-gated chloride channels that exist as pentameric protein complexes which are assembled from ligand-binding α subunits and a structural γ polypeptide [2, 3]. At the postsynaptic membrane, glycine receptors are clustered by interaction with the tubulin-binding protein ► **gephyrin** [3]. ► **Hyperkplexia**, a human neurological disorder characterized by exaggerated startle responses and an increased muscle tone, is associated with mutant alleles of the glycine receptor $\alpha 1$ and γ subunit genes, *GLRA1* and *GLRB*. Likewise, homologous disease states exist in

mutant mouse lines carrying mutations of glycine receptor $\alpha 1$ and γ subunit genes [2].

Basic Characteristics

The Glycinergic Synapse and its Constituents

Glycinergic synapses show a widespread distribution throughout the CNS [1–3]. Glycine-mediated inhibition underlies the modulation of brain stem reflexes and the segmental regulation of spinal motoneurons by small interneurons including the Renshaw cells. The neurotransmitter pool of glycine is derived from both metabolic precursors and reuptake from the synaptic cleft. Synthesis of glycine from its precursor serine is catalyzed by the mitochondrial isozyme of ► **serine-hydroxymethyltransferase**, which is dependent on tetrahydrofolate and pyridoxal phosphate. Finally, glycine is degraded to CO₂ and ammonia by the glycine cleavage system, a mitochondrial enzyme complex. Mutations of its genes cause nonketotic hyperglycinemia, a devastating neonatal disease characterized by lethargy, seizures, and mental retardation in surviving patients. In the presynaptic terminal, the release fraction of glycine is stored in small synaptic vesicles. Vesicular loading of glycine is mediated by the vesicular inhibitory amino acid transporter (VIAAT or vesicular GABA transporter/VGAT), which is also involved in synaptic vesicular storage of GABA. Inhibitory miniature potentials recorded from rat motoneurons are consistent with the presynaptic vesicular release of the neurotransmitter into the synaptic cleft. The exocytotic release of glycine is a highly regulated process, where the vesicle protein synaptobrevin plays a pivotal role for vesicle fusion with the presynaptic membrane.

► **Tetanus**, a disease caused by the anaerobic, spore-forming rod *Clostridium tetani*, is associated with a presynaptic block of glycine release. The clostridial protein, tetanus toxin, possesses a protease activity which selectively degrades synaptobrevin. Consistent with a loss of glycinergic inhibition, the hypertonic motor symptoms of tetanus resemble strychnine intoxication. Once released into the synaptic cleft, glycine is rapidly taken up by sodium-dependent transporters characterized by distinct regional distributions. The transporters GLYT1a and GLYT1b represent splice variants of the same gene, differing in N-terminal structure. Both variants show a widespread distribution throughout the CNS, with GLYT1a predominating in the grey matter and GLYT1b in the white matter. In contrast, the transporter GLYT2 colocalizes with the strychnine-sensitive glycine receptor, suggesting a role in the termination of glycinergic inhibition.

Glycine Receptor Structure and Genetics

Receptor-binding of glycine induces the opening of an intrinsic anion channel highly selective for chloride and bicarbonate [2–4]. Depending on the low reversal

potential for chloride that prevails in many neurons of the mature CNS, this elicits inward chloride currents and a postsynaptic hyperpolarization. Glycine receptors are derived from a family of highly homologous subunit genes. This group of genes is part of the superfamily of ligand-gated ion channels which also includes the nicotinic acetylcholine, GABA_A, and 5-HT₃ receptors. Glycine receptor subunits assemble into pentameric channels thought to form a rosette-like arrangement surrounding a central ion pore. Glycine receptor isoforms are characterized by distinct developmental and regional expression patterns. The adult isoform, GlyR_A, is an oligomeric protein composed of ligand-binding $\alpha 1$ and structural γ subunits. The neonatal isoform, GlyR_N, prevails in newborn rodents and is replaced by the adult type GlyR_A within two weeks postnatally. The GlyR_N protein appears to be a homooligomer composed of $\alpha 2$ subunits. In addition to $\alpha 3$ and $\alpha 4$ subunits encoded by distinct genes, further complexity of α subunits results from alternative pre-mRNA splicing [3].

Glycine receptor α and γ subunit variants are characterized by transmembrane topologies common to the superfamily of ligand-gated ion channels [2, 3]. A large, N-terminal extracellular domain, which in the mature $\alpha 1$ subunit comprises 220 amino acid residues, is followed by four transmembrane regions (TM1 to TM4) spanning the postsynaptic membrane. A fifth hydrophobic region preceding the mature protein represents the cleavable signal peptide. Displaying a rare ability among receptor channel polypeptides, glycine receptor α subunits are self-sufficient in creating homomeric receptor channels when subjected to recombinant expression. Recombinant glycine receptor variants faithfully reproduce pharmacological characteristics of their native counterparts from mammalian CNS. Attempts to elucidate structure–function relationships of glycine receptor subunits have led to the identification of structural motifs involved in distinct steps of ligand-gated ion conductance: (i) As deduced from radioligand-binding studies and whole-cell current recordings, glycine and strychnine bind to partially overlapping, but not identical, sites on the receptor. Determinants of ligand-binding and agonist-antagonist discrimination have been assigned to the N-terminal domain, where two stretches of amino acid residues preceding the TM1 domain were identified to contribute to the ligand-binding pocket. A recombinant switch in aromatic hydroxyl groups flanking position $\alpha 1(160)$ generated a γ -alanine receptor responsive to GABA. (ii) Domains involved in anion translocation include the TM2 region as well as the short loops flanking TM2 at the intracellular and extracellular faces of the plasma membrane. Receptor-gating, i.e., the open–close transition of the ion channel, has been interpreted as an intramolecular motion of TM2, where the flanking loops act as hinges

for the conformational transition. While two anion binding sites within the glycine receptor channel have been postulated from electrophysiological analysis, the pathway of chloride permeation across the membrane still awaits elucidation. ► **Desensitization** of glycine receptor ion channels is affected by intracellular determinants positioned within the short loop between TM1 and TM2, as well as within the large loop connecting TM3 and TM4. In particular, the splice variants $\alpha 3K$ and $\alpha 3L$ which differ in a motif located within the TM3–TM4 loop, also differ in desensitization behavior. (iii) Receptor assembly and heteropentameric subunit stoichiometry are governed by motifs, in particular an eight amino acid sequence, residing within the N-terminal extracellular regions of the receptor subunits.

Excitatory Action of Glycine Receptors in Developing CNS

In the embryonic CNS and in dorsal root ganglia, the neuronal reversal potential for chloride is above the membrane potential. Under these conditions, opening of glycine-associated chloride channels will result in depolarizing currents and subsequent excitation, once the neuronal threshold for formation of action potentials is exceeded. During a short postnatal period, glycine receptors are abundantly expressed in rat cerebral cortex which, by immunological criteria, correspond to the GlyR_N isoform. Activation of the cortical glycine receptors results in excitatory impulses and appears to be mediated by nonsynaptically released taurine [2]. The functional role of glycine receptor mediated excitation in the developing neocortex remains to be established.

Postsynaptic Clustering by Gephyrin

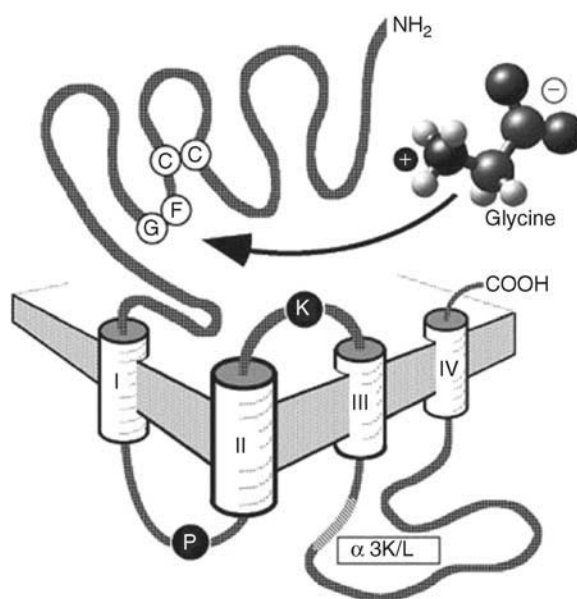
Glycine receptors associate with gephyrin, a tubulin-binding protein involved in the formation of postsynaptic receptor clusters [3]. Gephyrin is thought to form a submembraneous protein scaffold that dramatically reduces lateral diffusion of the receptor complexes. This interaction increases receptor life-time by stabilizing the protein remaining in its postsynaptic location in the plasma membrane. Receptor stabilization is activity-dependent, resulting in a loss of receptors under conditions of reduced glycinergic transmission, as induced by application of strychnine. Interaction of gephyrin and glycine receptor subunits occurs by means of an 18 amino acid residue motif located within the intracellular loop between TM3 and TM4. Gephyrin also interacts with GABA_A receptors, potentially via the GABA_A receptor associated protein (GABARAP). As additional components of the postsynaptic protein scaffold, phosphatidylinositol 3,4,5-trisphosphate-binding proteins have been identified, including collybistin and profilin. However, gephyrin also serves dual functions apparently beyond synaptic

organization, as it contributes to biosynthesis of the molybdenum cofactor, an essential coenzyme of a variety of dehydrogenases.

Disease Mechanisms

Based on its clinical resemblance to subconvulsive strychnine poisoning, glycine receptor dysfunction has long been considered a candidate mechanism of hypertonic motor disorders [1, 2]. As exemplified in the spontaneous mouse mutants spastic, spasmodic, and oscillator, glycine receptor defects result in hereditary neurological disorders. In the spastic mouse, the intronic insertion of a LINE-1 transposable element into the γ subunit gene *Glyrb* results in aberrant splicing and a consecutive loss of receptors. Consistent with a numerical receptor defect, the spastic phenotype is rescued by a transgene expressing γ subunit mRNA. The spasmodic mouse carries a missense mutation of the $\alpha 1$ subunit gene, *Glra1*(A52S) that diminishes agonist affinity. The spasmodic locus is linked by synteny homology to the human chromosomal region 5q31.3 carrying the human *GLRA1* gene. In the oscillator mutant, a microdeletion within the *Glra1* gene causes a complete loss of the glycine receptor isoform GlyR_A, resulting in lethality. During development, the postnatal appearance of all of these mutant phenotypes coincides with the switch from the unaffected neonatal (GlyR_N) to the diminished adult receptor isoform, GlyR_A.

Hyperekplexia (startle disease, stiff baby syndrome) is a congenital human motor disorder that follows autosomal-recessive as well as dominant modes of inheritance [2]. Affected patients exhibit an exaggerated startle response and increased muscle tone. Hyperekplexia is associated with a variety of *GLRA1* and *GLRB* mutant alleles that affect glycine receptor affinity and ion conductance. In the disease associated $\alpha 1$ subunit variants, the amino acid positions mutated cluster near segment TM2. In particular, the hyperekplexia allele $\alpha 1$ (P250T) predicts a substitution in the cytoplasmatic loop TM1–TM2 (Fig. 1). Recombinant $\alpha 1$ (P250T) channels show reduced chloride conductance and enhanced desensitization, defining an intracellular determinant of channel-gating. In contrast, the mutation $\alpha 1$ (K276E) situated within the extracellular loop TM2–TM3, is without effect on channel conductance, but almost exclusively affects gating. Hyperekplexia mutations of *GLRA1* also give clues to glycine receptor regulation in the human: In several cases of recessive hyperekplexia, homozygosity for a null allele of *GLRA1* was found, consistent with a complete loss of gene function. Born to consanguineous parents, the affected children displayed relatively mild symptoms despite this “knockout” situation. In contrast to lethality of the null allele in homozygous oscillator mice, the complete loss of the $\alpha 1$ subunit is tolerated in



Glycine Receptors. Figure 1 Transmembrane topology of glycine receptor α subunits. Positions of amino acid exchanges $\alpha 1$ (P250T) and $\alpha 1$ (K276E) associated with the human neurological disorder hyperekplexia are as indicated by filled circles, amino acid residues involved in ligand binding and discrimination are indicated by open circles. The glycine receptor subunit variant $\alpha 3L$ carries a cytoplasmic insertion sequence (box) which is generated by alternative splicing. This insertion is missing from the splice variant $\alpha 3K$.

the human. This suggests that either the loss of glycine receptors is effectively compensated or that subunit regulation substantially differs among these species [5].

Drugs

The inhibitory glycine receptor still lacks a therapeutic pharmacology. The agonistic properties of glycine are imitated by a series of structurally related amino acids [1,2]. In spinal neurons, the relative potency of these agonists decreases in the order of: glycine > γ -alanine > taurine > α -alanine > serine. In contrast, the structurally related amino acid GABA is not an agonist at glycine receptors. Recombinant $\alpha 1$ subunit receptors respond to this group of glycinergic amino acid agonists, while recombinant $\alpha 2$ subunit receptors are preferentially activated by glycine and barely respond to γ -alanine and taurine. The convulsant alkaloid strychnine is a high affinity antagonist ($K_D \approx 10$ nM) of receptor binding by glycine ($K_D \approx 10$ μ M). Consistent with the physiology of glycinergic synapses, sublethal strychnine poisoning causes motor disturbances, e.g., increases in muscle tone and hyperreflexia. Further symptoms include alterations of sensory, visual, and acoustic perception. As a result of dysinhibition in auditory and motor centers, strychnine

generates excessive startle responses, while higher doses lead to convulsions and death. Glycine displaceable binding of [^3H]strychnine is a highly specific probe of the glycine receptor. High affinity binding of [^3H]strychnine has been demonstrated to spinal cord, sensory, and acoustic ganglia of the brain stem as well as to retina. Symptoms of strychnine intoxication correlate to the dysfunction of those CNS regions displaying high [^3H]strychnine binding. In addition, numerous drugs and toxins including muscimol analogues, benzodiazepines, convulsant steroids, and picrotoxinin have been shown to exert strychnine-like effects, yet at significantly higher concentrations.

Glycine receptor function is modulated by alcohols and anesthetics [4]. Amino acid residue $\alpha 1$ (S267) is critical for alcohol potentiation, as mutation to small residues (Gly, Ala) enhance, and mutation to large residues (His, Cys, Tyr) diminish the ethanol effect. Glycine receptor modulation by Zn^{2+} involves structural determinants located within the large N-terminal domain. Additional glycinergic modulators include neuroactive steroids and the anthelmintic, ivermectin, which activates glycine receptors by a novel, strychnine-insensitive mechanism.

► GABAergic System

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glycogen by adding UDP-glucose. Phosphorylation of GS by GSK3 leads to inactivation of GS. GSK3 is a substrate of the protein kinase Akt. Stimulation of Akt by insulin leads to phosphorylation of GSK3, to inhibition of its kinase activity, and consequently to activation of GS and glycogen synthesis. Recently, a role of GSK3 in the regulation of insulin-dependent gene expression has also been described. GSK3 called zeste-white/shaggy in *Drosophila*, plays a key role in the degradation of β -catenin in the absence of Wnt signaling and is a promising target for pharmacological manipulation of Wnt signaling pathways in both cancer and other disease contexts. There are two isoforms (α and β), with GSK3 β being most important in Wnt signaling.

► Insulin Receptor

► Wnt Signaling

Glycopeptide Antibiotics

Glycopeptide antibiotics, a group which includes vancomycin and teicoplanin, are primarily active against gram-positive bacteria, including methicillin-resistant staphylococci. They have also been used against resistant enterococci. However, resistance against glycopeptide antibiotics is now emerging rapidly world-wide. Glycopeptide antibiotics inhibit the synthesis of the cell wall in sensitive bacteria by binding with high affinity to the D-alanyl-D-alanine terminus of cell wall precursor units. The drug is bactericidal for dividing microorganisms. Enterococcal resistance to vancomycin results from the alteration of the D-alanyl-D-alanine target to D-alanyl-D-lactate or D-alanyl-D-serine, which bind vancomycin poorly.

► β -Lactam Antibiotics

► Ribosomal Protein Synthesis Inhibitors

► Quinolones

► Microbial Resistance to Drugs

Glycogen Synthase Kinase 3

Synonym

GSK3

Definition

GSK3 phosphorylates glycogen synthase (GS), the key enzyme for glycogen synthesis which builds up the

Glycoprotein IIb/IIIa Receptor Antagonists

► Anti-Integrins, Therapeutic and Diagnostic Implications

► Antiplatelet Drugs

Glycoproteins

Glycoproteins are proteins to which one or more oligosaccharide chains are covalently linked.

►Glycosylation

Glycosaminoglycan

A glycosaminoglycan is any one of a number of nonbranching, sulfated polysaccharides, consisting of repeating disaccharide units comprised of a uronic acid moiety (glucuronic or iduronic acid) and an amino sugar (glucosamine or galactosamine) that are variably O- and N-sulfated and N-acetylated, and polydisperse (variable chain length). For example, heparin consists of alternating uronic acid (either glucuronic or iduronic acid moieties that are variably 2-O-sulfated) and glucosamine (variably 2-N-sulfated, 3-O-sulfated, 6-O-sulfated, and/or 2-N-acetylated).

►Anticoagulants

Glycosides, Cardiac

►Cardiac Glycosides

Glycosylation

Most secreted proteins and many membrane proteins are ►glycoproteins. There are two main types of glycosylation: N-glycosylation, in which the oligosaccharide is attached to an asparagine residue, and O-glycosylation, in which the oligosaccharide is attached to a serine or threonine residue. Glycosylation is one of the most ubiquitous forms of posttranslational modification. The assembly takes place in a stepwise fashion primarily in the ►endoplasmic reticulum and Golgi apparatus. Glycosylation encodes information for specific molecular recognition affecting also protein folding, stability, and pharmacokinetics.

►Intracellular Transport

►Protein Trafficking and Quality Control

Glycosylphosphatidylinositol Anchor (GPI Anchor)

GPI anchoring is a posttranslational modification occurring in the ►endoplasmic reticulum where preassembled GPI anchor precursors are transferred to proteins bearing a C-terminal GPI signal sequence. The GPI anchor precursors are synthesized in the endoplasmic reticulum by sequential addition of sugar and other components to phosphatidylinositol. Protein GPI anchors are ubiquitous in eukaryotic cells. In mammalian cells, GPI anchored proteins are often found in lipid rafts which are subdomains of the plasma membrane, containing various signaling components.

►Lipid Modifications

Glycylcyclines

Glycylcyclines are a new generation of tetracyclines (e.g. tigilcycline), which have been developed to overcome problems of resistance to common tetracyclines.

►Ribosomal Protein Synthesis Inhibitors

Glypiation

►Lipid Modifications

GM-CSF

Granulocyte-macrophage-CSF.

►Hematopoietic Growth Factors

GM2-gangliosidosis

GM2 gangliosidosis (also known as Tay–Sachs disease) is a rare disorder caused by mutations in the gene encoding the lysosomal, heterodimeric α -hexosaminidase

A (Hex A) catalyzing the biodegradation of gangliosides. Accumulation of gangliosides in lysosomes primarily affects brain neurons. In the early onset form of the disease, children become blind, deaf, and unable to swallow. Children die usually before the age of three. A late onset form of the disease occurs in young adults and is usually nonfatal.

► Protein Trafficking and Quality Control

GMP, Cyclic

- Cyclic Guanosine Monophosphate
- Guanylyl Cyclases
- Smooth Muscle Tone Regulation

GnRH

- Gonadotropin-releasing Factor/Hormone (GnRH)

Gold Compounds

Gold compounds such as sodium aurothiomalate or auranofin are used to treat chronic inflammatory diseases like rheumatoid arthritis. The anti-inflammatory effects develop slowly, with maximum action occurring after 3–4 months. The exact mechanism of action is not understood. However a variety of inflammatory processes such as mitogen-induced lymphocyte proliferation, the activation of lysosomal enzymes, the production of O₂ metabolites, the chemotaxis of neutrophils or the induction of various cytokines have been shown to be inhibited by gold compounds. Gold compounds belong to the category of ► **disease-modifying anti-rheumatic drugs (DMARDs)**.

- Rheumatoid Arthritis

Golgi Apparatus

The Golgi apparatus is a stack of flattened vesicles that functions in posttranslational processing and sorting

of proteins. The Golgi apparatus receives proteins from the endoplasmic reticulum via the endoplasmic reticulum/Golgi intermediate compartment and directs them to endosomes, lysosomes or the plasma membrane. It is organized into a number of stacks of disc like compartments (cisternae).

- Intracellular Transport
- Protein Trafficking and Quality Control

Gonadorelin

- Gonadotrophin-releasing Factor/Hormone (GnRH)

Gonadotropin-releasing Factor/Hormone (GnRH)

GnRH is derived by proteolytic processing of a 92-amino acid precursor peptide to produce mature GnRH, a decapeptide. GnRH is released from neurons in the hypothalamus. GnRH release is intermittent and is governed by a neural pulse generator that is located in the mediobasal hypothalamus, that controls the frequency and amplitude of GnRH release. The GnRH pulse generator is active during fetal life and for about one year after birth, thereafter its activity decreases but increases again during puberty. The intermittent release of GnRH is crucial for the proper synthesis and release of the gonadotropins, which are also released in a pulsatile manner. The gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are synthesized and secreted by gonadotrophs, which make up about 20% of the hormone-secreting cells in the anterior pituitary. GnRH is the main stimulus which induces secretion of LH and FSH. The continuous administration of GnRH, in contrast to the physiological intermittent action, leads to desensitization and down-regulation of GnRH receptors on pituitary gonadotrophs. This action forms the basis for the clinical use of long-acting GnRH analogs that suppress gonadotropin secretion. These compounds transiently increase LH and FSH secretion, but eventually desensitize gonadotrophs to GnRH, and thereby inhibit gonadotropin release. Synthetic GnRH is termed “gonadorelin”. GnRH and a variety of analogs with agonist activity like buserelin, leuporelin, goserelin or nafarelin can be given in a continuous fashion in order to achieve

gonadal suppression through the decreased production of FSH and LH. Gonadal suppression may be desirable to treat endometriosis, precocious puberty, sex hormone-dependent cancers (e.g. advanced prostatic cancer), or hirsutism due to the polycystic ovary syndrome.

► Contraceptives

Gonadotropins

The pituitary hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) together with the placental hormone chorionic gonadotropin are collectively called gonadotropins. They are large, glycosylated heterodimers composed of a common α -subunit and a hormone-specific γ -subunit. The main targets are the ovary and testes.

► Gonadotropin-releasing Factor/Hormone (GnRH)
► Contraceptives
► Aromatase

Gout

Gout is the consequence of hyperuricemia and is characterized by uric acid deposits in joints, bursae, tendons, kidney, and urinary tract. In the initial stage, gout is characterized by asymptomatic hyperuricemia. In the second stage, the disease manifests itself by acute gouty arthritis. The third (intercritical) stage is asymptomatic, and the fourth stage is characterized by progressive uric acid desposits in joints, bursae, tendons, kidney, and urinary tract.

► Anti-gout Drugs

GPCRs

► G-protein-coupled receptors

GPI Anchor

Glycosylphosphatidylinositol Anchor.

► Lipid Modifications

GPR30

GPR30 is a G protein coupled estrogen receptor that is thought to mediate nongenomic actions of estrogen, which are initiated from the cell membrane.

► G-protein-coupled Receptor
► Selective Sex Steroid Receptor Modulators

G-protein-coupled Receptor Kinases (GRKs)

G-protein-coupled receptor kinases (GRKs) are a family of enzymes that catalyze the phosphorylation of threonine or serine residues on G-protein-coupled receptors. Characteristically, GRKs only phosphorylate the ligand-activated form of the receptors. Phosphorylation by GRKs usually leads to impaired receptor/G-protein coupling.

► G-protein-coupled Receptors
► Tolerance and Desensitization

G-protein-coupled Receptors

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Synonyms

Seven transmembrane helix receptors; Heptahelical receptors; Serpentine receptors

Definition

G-protein-coupled receptors (GPCRs) are a large family of plasma membrane receptors. Upon binding its agonist, a GPCR activates an intracellular heterotrimeric guanine nucleotide regulatory protein (G protein). The activated G protein modulates the activity of one or more enzymes or ion channels. G protein independent signaling pathways have also been identified for several receptors.

Basic Characteristics

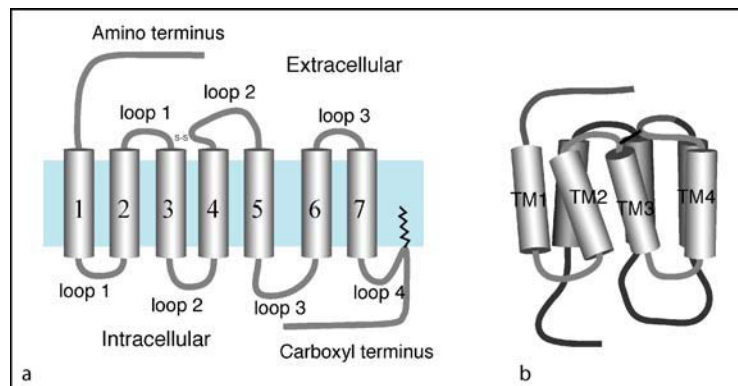
Cells receive much of the information about their external environment by way of receptor proteins that span the plasma membrane. GPCRs are the largest family of plasma membrane receptors. They mediate response to the majority of hormones and neurotransmitters as well as the senses of sight, smell, and taste. These receptors have in common a seven transmembrane topology and functional interactions with heterotrimeric guanine nucleotide binding proteins (G proteins). GPCRs respond to a large variety of stimuli ranging from photons, ions, amino acids, and small organic molecules to peptide and protein hormones.

Analysis of the human genome reveals over 800 unique GPCRs [1]. The functional role for many of these GPCRs has not yet been determined and these have been designated as orphan GPCRs. GPCRs can be divided into five families based on sequence homology: the rhodopsin family, the secretin receptor family, the glutamate receptor family, the adhesion receptor family, and the frizzled/TAS2 receptor family. The rhodopsin family is by far the largest with over 700 members. Of these 460 belong to the subfamily of odorant receptors, and the rest are receptors for hormones and neurotransmitters.

GPCRs as a group constitute the largest family of targets for pharmacological intervention. They are critically involved in virtually every physiological system. A partial list of natural GPCR ligands includes protons, calcium, glutamate, GABA, acetylcholine, histamine, GTP/ATP, adenosine, cAMP, melatonin, epinephrine, serotonin, and dopamine. Peptide hormone GPCR activators include angiotensin, vasopressin, bradykinin, calcitonin, FSH, glucagon, somatostatin and a host of chemokines, opioids, and cannabinoids. Olfactory and gustatory sensory transduction involves GPCRs responsive to a large array of odorants and pheromones, and vision depends on the light-activated ligand retinal covalently bound to its own GPCR rhodopsin.

Receptor Structure

The structure of only one GPCR, bovine rhodopsin, has been solved at high resolution; however, it is thought that all GPCRs have the same core structure (Fig. 1) consisting of seven transmembrane (TM) spanning domains with an extracellular amino terminus and an intracellular carboxyl terminus [1]. The structures of GPCRs diverge most in the amino terminus, the carboxyl terminus, and the intracellular loop between TM5 and TM6. Amino termini are frequently glycosylated and range in size from 7 to 595 amino acids. The intracellular carboxyl terminus is typically tethered to the membrane by a lipid modification such as palmitoylation and ranges in size from 12 to 359 amino acids. The carboxyl terminus and the intracellular loop between TM5 and TM6 often contain sites for phosphorylation by one or more regulatory kinases such as protein kinase A, protein kinase C, or a member of the GPCR kinase (GRK) family. A disulfide bond between two highly conserved cysteines links the second and third extracellular loops of most GPCRs.



G-protein-coupled Receptors. Figure 1 Diagrams of the structural characteristics of G-protein-coupled receptors (GPCRs). (a) The secondary structure of a generic GPCR showing the seven membrane spanning domains and connecting loops. (b) A cartoon of the predicated three-dimensional structure of a generic GPCR based on the structure of rhodopsin. These diagrams were taken from [1].

Ligand Binding

The location of the agonist binding site is highly variable (Fig. 2). Monoamine hormones such as catecholamines, dopamine, serotonin, and acetylcholine bind within the TM core (Fig. 2a). Small peptide hormones bind to the amino terminus, the extracellular loops between TM domains and within the TM core (Fig. 2b). Large amino terminal domains form the binding site for glycoprotein hormones (such as follicle stimulating hormone) (Fig. 2c), as well as for ions (the Ca^{2+} -sensing receptor, Fig. 2d) and the neurotransmitters glutamate and GABA. Finally, in protease-activated receptors, the agonist is generated by proteolytic cleavage of the amino terminus of the receptor (Fig. 2e).

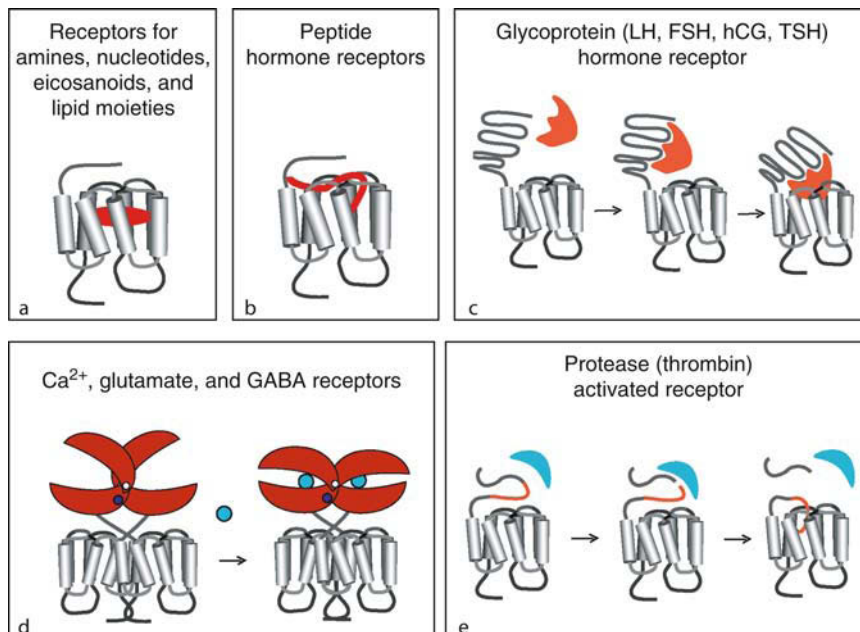
G-protein Coupling Domains

Mutagenesis studies have identified multiple sites of interaction between GPCRs and their cognate G proteins. These include the intracellular loop 2 between TM3 and TM4, the intracellular loop 3 between TM 5 and TM 6, and loop 4 formed between TM 7 and the lipid modification on the proximal carboxyl terminus (Fig. 1). Agonist binding (Fig. 3a) is thought to lead to subtle changes in the arrangement of the TM domains [2]. These conformational changes are transmitted to the associated G protein. In an interaction that has yet to be fully characterized, this movement triggers a G protein heterotrimer ($\text{G}\alpha\beta\gamma$) to dissociate into $\text{G}\alpha$ and $\text{G}\beta\gamma$ subunits, thereby unmasking interactive domains on both of the freed G protein subunits and on the receptor itself. Both dissociated $\text{G}\alpha$ and $\text{G}\beta\gamma$ subunits

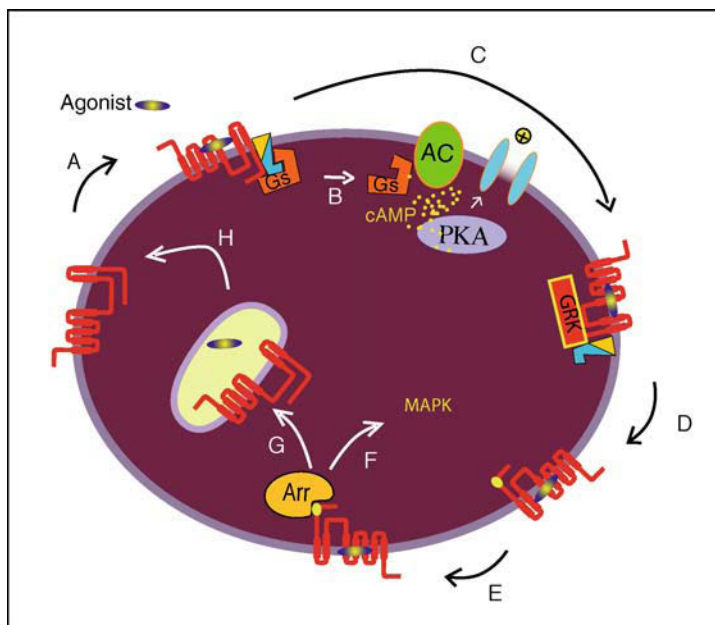
are capable of modulating a variety of effector systems including adenylyl cyclase, phospholipase C and ion channels (Fig. 3b). Recent studies suggest that some GPCRs may also signal through G protein independent pathways [3].

Pharmacologic Modulation of Receptor Activity

Ligands that bind to GPCRs display a broad spectrum of activity ranging from full **agonist**, to partial agonists, to neutral **antagonist**, to **inverse agonists**. Some GPCRs are capable of activating G proteins even in the absence of a ligand. This ligand-independent activity is often referred to as basal or constitutive activity. Inverse agonists suppress this basal activity while neutral antagonists do not alter basal activity. Full and partial agonists increase G protein activation above that attributed to the basal, ligand-independent activity. Perhaps the most widely accepted model used to describe ligand modulation of GPCRs is the extended ternary complex model. In its simplest form, this model proposes that the receptor exists in two functionally distinct states in equilibrium: the inactive (R) and the active (R^*) state. In the absence of ligands, the level of basal receptor activity is determined by the equilibrium between R and R^* . The efficacy of ligands is thought to be a reflection of their ability to alter the equilibrium between these two states. Full agonists stabilize R^* while inverse agonists stabilize R. The majority of natural hormones and neurotransmitters act as agonists for their associated receptors. However, there is



G-protein-coupled Receptors. Figure 2 Diagram illustrating the binding sites for different families of hormones and neurotransmitters on their receptors.



G-protein-coupled Receptors. Figure 3 Signaling cycle for the β_2 adrenergic receptor (β_2 AR), a prototypical GPCR. (a) The agonist (adrenaline) binds to the β_2 AR, which leads to activation of the associated G protein (G_s). (b) $G_{s\alpha}$ dissociates and activates adenylyl cyclase (AC), which converts ATP to cyclic AMP (cAMP). cAMP activates protein kinase A (PKA) which phosphorylates and activates a channel. (c) Following G_s activation, a G-protein-coupled receptor kinase (GRK) associates with the agonist-occupied receptor and $G_{\beta\gamma}$. (d) GRK phosphorylates the receptor. (e) Arrestin binds to the phosphorylated receptor blocking further interactions with G_s . (f) Arrestin also promotes signaling through MAPK and other pathways independent of G protein activation. (g) Arrestin promotes internalization of the receptor into an endosomal compartment where the phosphate is removed. (h) The dephosphorylated receptor is recycled back to the plasma membrane.

substantial evidence that the agouti-related peptide behaves as an antagonist or possibly an inverse agonist for the melanocortin receptor.

While the simple two-state model is able to explain many aspects of GPCR function, there is a growing body of evidence that GPCRs are conformationally complex molecules having ligand-specific receptor states [2]. Thus, partial agonists induce an active conformation that is distinct from full agonists. Many GPCRs are known to activate more than one heterotrimeric G protein subtype. The efficacy profile for a panel of GPCR ligands may differ for different G protein subtypes. The term stimulus trafficking refers to the ability of specific ligands to preferentially direct GPCR signaling to one G protein subtype.

GPCR Dimers

There is abundant experimental evidence suggesting that many (if not most) GPCRs exist as homodimers and/or heterodimers [4]. The functional significance of dimers and their role in G protein activation remains to be determined for most GPCRs. Experimental evidence suggests that dimers may play roles in processing and maturation of newly synthesized receptor protein. Heterodimers may contribute to pharmacologic

diversity and heterologous regulation of one receptor subtype by another. Dimers are required for G protein activation by members of the Glutamate receptor family; however, rhodopsin and the β_2 adrenoceptor have been shown to efficiently activate G proteins as monomers. The requirement for GPCR dimers is most convincing for heterodimers such as the functional GABA_B receptor formed by a GBR1/GBR2 heterodimer. The GBR2 behaves as a chaperone bringing the GBR1 to the cell surface. However, while GBR1 contains the main determinants for ligand binding, GBR2 is the major site of G protein coupling.

Regulation of GPCR Function

GPCR function has been shown to be regulated by several different mechanisms. The number of receptors on the plasma membrane may be regulated by transcription, mRNA stability, biosynthetic processing, and protein stability. In addition, the function of receptors in the plasma membrane can be influenced by regulatory phosphorylation and by association with other proteins that determine the subcellular location of receptors relative to other signaling molecules.

One of the most well characterized regulatory pathways for GPCRs is that mediated by the GRK

family [5]. After a signaling event, a multistage desensitization process begins with the agonist-dependent phosphorylation by a GRK of one or more serines or threonines located on intracellular domains of the receptor, particularly in the carboxyl terminus and the third intracellular loop (between TM5 and TM6) (Figs. 3c and d). This phosphorylation promotes the recruitment of an arrestin (Fig. 3e). Arrestins are a family of molecules that bind to agonist-occupied, phosphorylated GPCRs and interfere with G protein coupling. Arrestins may mediate receptor internalization (Fig. 3f) by way of clathrin coated pits or other mechanisms. Internalized receptors may be targeted for degradation (downregulation) or, after being dephosphorylated to restore functionality, they may be redeployed at the cell surface (Fig. 3g). In addition to GRKs, protein kinase A and protein kinase C have been shown to play roles in the desensitization of several GPCRs.

Drugs

Limited list (Trade Name)

Albuterol (Ventolin[®])
 Alprenolol
 Amthamine
 Antihistamines [class]
 Atenolol (Tenormin[®])
 Baclofen (Lioresal[®])
 γ -Blockers [class]
 Betaxolol
 γ -Funaltrexamine
 Bisoprolol
 Bromocriptine
 Caffeine
 Candesartan (Atacand[®])
 Cannabinoids
 Carbamazepine
 (Tegretol[®])
 Carvedilol (Coreg[®])
 Cimeterol
 Cimetidine (Tagamet[®])
 Cirazoline
 Clemastine
 Clenbuterol
 Clobenpropit
 Clocinnamox
 Clonidine (Catapres[®])
 Clozapine
 Corynanthine
 DAMGO
 Deltorphan
 Dihydroxidine
 Dihydroergocristine
 Dihydroergotamine
 Dilazep

Dimaprit
 Dobutamine (Dobutrex[®])
 Doxazosin (Cardura[®])
 Doxepin
 Epinephrine (Adrenalin)
 Famotidine (Pepcidine)
 Flavorings
 Fluorobenzylpiperone
 Fluoxetine (Prozac[®])
 Formoterol
 Guanabenz
 Guanfacine
 Haloperidol
 Hydrocodone (Vicodin[®])
 Hyoscyamine (Levsin[®])
 Ibopamine
 ICI 118,551
 Ifenprodil
 Imetit
 Immepip
 Iodophenpropit
 Ipratropium (Atrovent[®])
 Irbesartan (Avapro[®])
 Ketotifen
 Loratadine (Claritin[®])
 Losartan (Cozaar[®])
 Mepyramine
 Metoprolol (Toprol-XL[®])
 Midazolam (Versed[®])
 Morphine
 Naftopidil
 Naloxonazine
 Naltriben
 Naltrindole
 Nizatidine (Axid[®])
 Opioids [class]
 Oxymetazoline
 Paroxetine (Paxil[®])
 Pimozide
 Pindolol
 Piribedil
 Practolol
 Prazosin
 Procaterol
 Pronethanol
 Propranolol (Inderal[®])
 Quinpirole
 Ranitidine (Zantac[®])
 Remoxipride
 Rilmenidine
 Salbutamol
 Salmeterol (Serevent[®])
 Scents
 Sotalol
 Sulpiride
 Sumatriptan (Imitrex, Imigran[®])

Tamsulosin (Flomax[®])
 Terazosin (Hytrin[®])
 Theophylline (TheoDur[®])
 Thioperamide
 Timolol (Blocadren[®])
 Tiotidine
 Tizanidine (Zanaflex[®])
 trans-Triprolidine
 Valsartan (Diovan[®])
 Ondansetron (Zofran[®])
 Zolantidine

- Table Appendix: Receptor Proteins
- Drug–Receptor Interaction
- Tolerance and Desensitization
- Transmembrane Signaling
- Prostanoids
- Adenosine Receptors
- Galanin Receptors

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G-proteins and Wnt Signaling

Definition

The topological resemblance of the ►Fz receptor to other seven-transmembrane receptors that couple to heterotrimeric G-proteins makes it logical to ask whether Fz receptors signal through ►heterotrimeric G-proteins as well. Evidence in favor of this hypothesis includes that stimulation of mammalian tissue culture cells by expressing one of the Wnt/Fz combinations implicated in Wnt/β-catenin-dependent signaling can be blocked by ►pertussis toxin and other G-protein inhibitors; in *Xenopus* embryos, ectopic expression of a ►Regulator of G-protein Signaling (RGS) protein antagonizes Wnt/β-catenin-dependent signaling and causes defects resembling those caused by ectopic

expression of other Wnt pathway inhibitors; reduced $G_{\alpha o}$ in *Drosophila* impairs wnt signaling in the developing fly wing. Similarly, over-expression of Wnt5a in vertebrate cells activates a non-β-catenin signaling pathway that is selectively blocked by several G-protein inhibitors, including pertussis toxin. Work with chimeric Fz receptors containing the extracellular and transmembrane domains of the β₂-adrenergic receptor suggests that G-proteins may couple to the intracellular domain of Fz receptors. These experiments implicate $G_{\alpha o}$ and $G_{\alpha q}$ for Fz receptors activating Wnt/β-catenin-dependent signaling, and $G_{\alpha o}$ and $G_{\alpha t2}$ for Fz receptors activating Wnt/non-β-catenin signaling. Nonetheless, the role of heterotrimeric G-proteins in Wnt signal transduction remains a point of contention in this signaling field.

- Wnt Signaling

Granulocyte-CSF (G-CSF)

- Hematopoietic Growth Factors

Granulocyte-macrophage-CSF (GM-CSF)

- Hematopoietic Growth Factors

Graves' Disease

Graves' disease is an organ-specific autoimmune disease. Antibodies against the ►TSH receptor mimic the action of TSH thereby causing stimulation of thyroid epithelial cells leading to hyperthyroidism. The specific cause of thyroid antibody production is not known at present. Susceptibility to Graves' disease is determined by genetic, environmental and endogenous factors.

- Antithyroid Drugs

Grb-2

Synonym

Growth Factor Receptor-bound Protein 2

Definition

Adaptor protein, containing one SH2 and two SH3 domains, which assembles signaling complexes at receptors. Particularly important for activation of the Ras-MAP kinase pathway.

- Adaptor Proteins
- Growth Factors

GRKs

- G-protein-coupled Receptor Kinases (GRKs)
- G-protein-coupled Receptors

Growth

Strictly speaking, growth refers specifically to an increase in mass. In biological contexts, and increase in mass is often coupled to (and is indeed often a prerequisite for) proliferation (an increase in cell number). Importantly, growth and proliferation are distinct processes that can be both physiologically and experimentally uncoupled.

- Growth Factors

Growth Factors

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Synonyms

Cytokines; Differentiation factors; Lymphokines; Mitogens

Definition

Growth factors are relatively small and stable, secreted or membrane-bound polypeptide ligands that mediate short-range cell-to-cell interactions. Growth factors and their cognate receptors function as a module that regulates various cellular processes, such as proliferation, differentiation, migration, or apoptosis. These modules are evolutionarily conserved, and their primary developmental function is determination of cell lineage through heterotypic cellular interactions. Many growth factors are versatile, promoting the appropriate biological outcome in several cell lineages, while others are more limited in scope. Growth factor expression is highly regulated, such that excessive growth factor activity is often associated with pathogenic hyperproliferation (e.g., cancer and psoriasis).

Classification

Growth factors are classified according to their structure and the family of receptors they activate. Whereas the definition of growth factors may broadly apply to lymphokines (e.g., interleukins; ILs) and cytokine-like members of the tumor necrosis factor (TNF) family, here we will consider only growth factors that bind to receptors of the receptor tyrosine kinase (RTK) superfamily. Growth factors bind such receptors with high affinity (in the low nanomolar range) and specificity, and they may be accordingly classified as ligands of a specific receptor subfamily [1] (see Table 1). For example, growth factors comprising an epidermal growth factor (EGF) motif bind to the ErbB subgroup of RTKs (also called type I RTKs). We will use this classification to elaborate on the shared mechanism of action of growth factors.

Mechanism of Action

Many growth factors are produced as prepropeptides, which are converted into biologically active forms, either soluble or membrane bound, by several steps of proteolytic cleavage and processing. These peptides, however, cannot penetrate the plasma membrane, and thus conveying the extracellular signal into the cell to promote the appropriate biological response entails the use of specialized receptors, traversing the plasma membrane [2]. The biochemical series of events that follows binding of a growth factor to the cell surface localized receptor, and culminating in cellular activation, is known as signal transduction (see Fig. 1).

The receptors have an extracellular ligand-binding domain, a hydrophobic segment traversing the plasma membrane, and an intracellular region which harbors a tyrosine kinase domain [2]. In most RTKs, the kinase is autoinhibited by the activation loop, which prevents the access of both ATP and substrate to the catalytic site. Ligand binding to the ectodomain induces large-scale conformational changes leading to receptor dimerization/

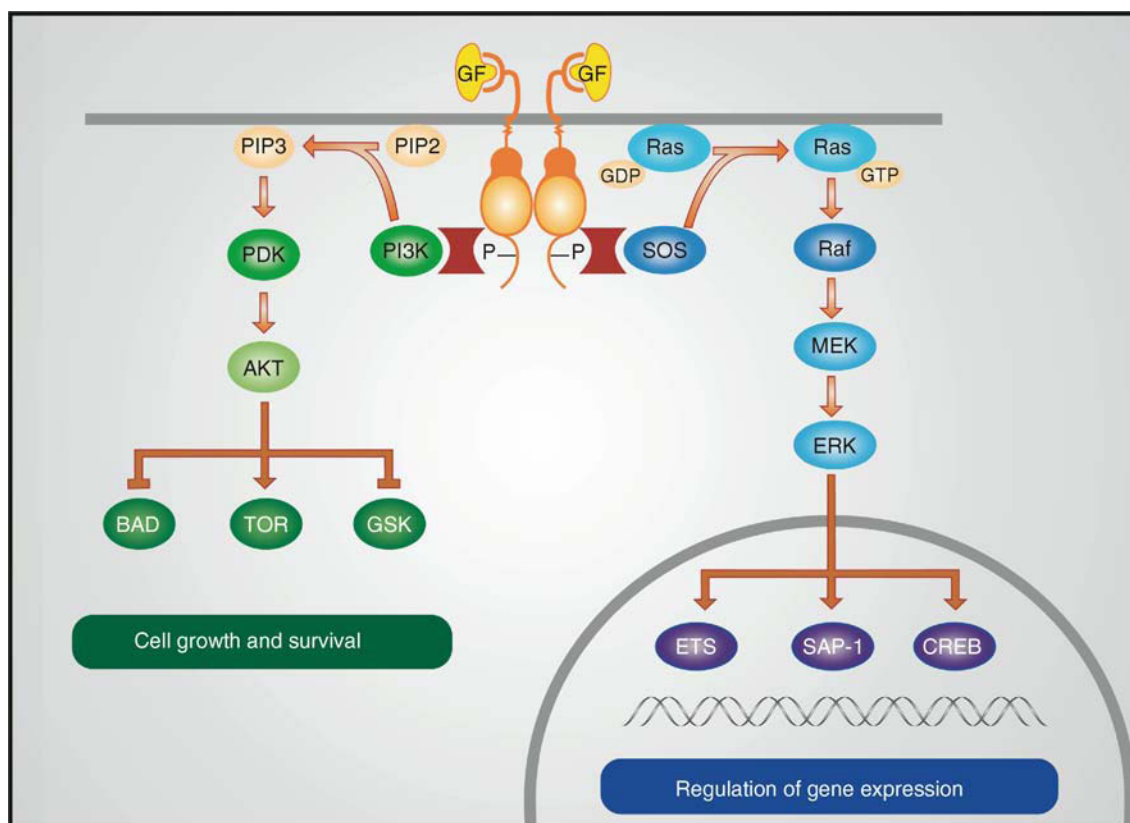
Growth Factors. Table 1 Summary of a number of growth factor families, the tyrosine kinase receptors they bind, and the major physiological consequences of their interactions

Growth factor family	Receptor tyrosine kinase	Major physiological functions
EGF family and neuregulins – EGF, TGF- α , Epiregulin, Amphiregulin, HB-EGF, Betacellulin, NRG1, NRG2, NRG3, NRG4, Epigen	ErbB family – ErbB1 (EGFR), ErbB2 (Neu, HER2), ErbB3, ErbB4	Morphogenesis, maintenance, and proliferation of epithelial, mesenchymal, and nerve tissues.
FGF (22 members): FGFs require heparan sulfate to activate their receptors	FGFR: Four members expressed as a number of splice variants	Proliferation of many cell types. Embryo patterning and organogenesis, bone development, angiogenesis
PDGF: Isoforms consist of homo- and heterodimers of A- and B-polypeptide chains and homodimers of C- and D-polypeptide chains	PDGFR: Consists of PDGFR α and β receptors	Embryonic development, particularly in the formation of the kidney, blood vessels, and various mesenchymal tissues. Proliferation of connective tissues, glial and smooth muscle cells
HGF: The ligands are heterodimers of A and B subunits linked by a disulfide bond	HGFR: Consists of three receptors: MET, SEA and RON	Motogenesis, morphogenesis, angiogenesis, and embryonic development
GDNF Family – GDNF, NRTN, ARTN, and PSPN	RET: Alternative splicing results in three isoforms	Required for enteric neuron development, kidney development, and spermatogenesis
VEGF family – VEGF-A, B, C, D, E and PLGF	VEGFR – VEGF receptor-1, 2, and 3	Formation and maintenance of vasculature
Angiopoietin family – Ang1, -2	TIE	Crucial for vessel stabilization
SCF: Due to splice variants there are soluble and membrane forms of SCF	KIT/SCFR	Hematopoiesis, gametogenesis, and melanogenesis
NGF family – NGF, BDNF, NT-3, and NT-4	TRK – TRK-A, B, C	Promotes neurite outgrowth and neural cell survival
Ephrins: Two classes, EphA and EphB, with a number of members within each class	EPHR: Two classes, EPHRA and EPHRB, with a number of members within each class	Patterning the developing hindbrain: Rhombomeres, axon pathfinding, and guiding neural crest cell migration

EGF, Epidermal growth factor; TGF- α , transforming growth factor- α ; AR, amphiregulin; HB-EGF, heparin binding EGF-like growth factor; NRG, neuregulin; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; HGF, hepatocyte growth factor; GDNF, glial-cell-line-derived neurotrophic factor; PLGF, placental growth factor; NRTN, neurturin; ARTN, artemin; PSPN, persephin; VEGF, vascular endothelial growth factor; SCF, stem cell factor; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-3,4, neurotrophin-3,-4; RET, rearranged during transfection; TIE, tyrosine kinase receptor in endothelial cells; Trk, tyrosine kinase receptor

oligomerization [3]. In cases where the receptors preexist as disulfide-linked dimeric receptors (e.g. receptors for insulin and insulin-like growth factors; IGFs), ligand binding causes a significant change in the juxtaposition of receptors. Conformational changes, however, are not confined to the ectodomain and transition to the ligand-bound active conformation is relayed across the plasma membrane and orients two kinase domains of two receptors to promote auto- and transphosphorylation of key tyrosines within the activation loop. This shifts the loop in a manner that releases its autoinhibitory effect (in contrast, phosphorylation of the loop is not a prerequisite for ErbB family kinase domain activation). Once activated, the kinase transfers phosphate groups to tyrosine residues of its own cytoplasmic tail and to nearby substrate proteins including dimer partners and other proteins participating in signal transduction.

Phosphorylated tyrosine residues of RTKs function as docking sites for a wide range of proteins containing ►Src homology, ►2 (SH2) or, ►phosphotyrosine-binding (PTB) domains. Among these proteins are cytosolic kinases (e.g., PI3K), lipases (e.g., PLC- γ), phosphatases (e.g., Dep1), and ubiquitin ligases (e.g., Cbl), which are recruited to the receptor either directly or through ►adaptor proteins (e.g., Shc and Grb-2) with multiple docking sites. Thus, a large multiprotein signaling complex is assembled at the membrane, with the receptor as the anchor. Recruited kinases phosphorylate additional proteins, further widening protein recruitment and branching of the signal, which is delivered to subcellular organelles, an example of which is the nucleus, where transcriptional regulators are activated and gene expression commences. ErbB-4, as well as some other RTKs, translocate a portion of the cytoplasmic domain into the nucleus, where the



Growth Factors. Figure 1 Growth factor-induced signal transduction pathways. Binding of growth factors (GFs) to their cognate receptors initiates the activation of intracellular signaling pathways, culminating in the nucleus, to propel and regulate key cellular processes. Demonstrated here are two of the best studied pathways: the pathway involving Ras and the MAPK cascade (*blue*), which activates multiple transcription factors (e.g., ETS, SAP-1, and CREB), which in turn regulate the expression of genes necessary for cell proliferation, differentiation, and migration; and the PI3K/Akt pathway (*green*), which modulates the activity of enzymes involved in the control of apoptosis (BAD) and metabolism (TOR, GSK). Both pathways are activated by binding of noncatalytic adaptor molecules (*brown*), which bind specific phosphorylated tyrosine residues of the receptor, thereby stimulating the first enzyme in a linear cascade of enzymatic reactions (see text for details).

catalytic fragment may participate in the regulation of gene expression.

Though the outcome of signaling events initiated by receptors within a family are distinct, some common themes have emerged. One of the signaling pathways common to many growth factor receptors is the route leading to stimulation of the mitogen-activated protein kinase, MAPK [4] (Fig. 1): ►Grb2, an ►SH2 and ►SH3 domain containing ►adaptor protein, is recruited to the phosphorylated receptor, either directly or through an ►adaptor protein called Shc. Upon receptor binding, Grb2 can interact through its ►SH3 domain with son of sevenless (SOS), a guanine nucleotide exchange factor. Translocation of SOS to a membrane proximal region facilitates its interaction with Ras, a membrane-anchored guanine-binding protein which SOS activates by exchanging GDP for GTP. Once Ras is in the GTP-bound form, it can recruit Raf, a cytosolic serine/threonine kinase, which in turn activates MEK, a dual

specificity protein kinase. MEK phosphorylates extracellular-regulated kinase (ERK) (also called MAPK) on both a threonine and a tyrosine residue within ERK's regulating domain, thereby activating it. Phosphorylated ERK translocates to the nucleus and activates multiple transcription factors, including ETS-1, ELK-1, and SAP-1. These transcription factors induce the expression of genes important for cell cycle progression and proliferation. In a similar manner, the GTP-bound form of Ras can also activate phosphoinositide 3'-kinase (PI3K), which generates specific inositol lipids. PI3K can be recruited directly by phosphorylated RTKs such as ErbB-3 (Fig. 1). A major target of PI3K lipid products is the protein kinase Akt (or protein kinase B; PKB). In quiescent cells, Akt resides in the cytosol in a low-activity conformation. Upon cellular stimulation, Akt is activated through recruitment to cellular membranes by PI3K lipid products and phosphorylation by 3'-phosphoinositide-dependent kinase-1 (PDK1). Akt is a serine/threonine

kinase that phosphorylates and inactivates proapoptotic proteins like BAD, procaspase 9, and FKHR-1, thereby promoting cell survival. Akt also induces proliferation by antagonizing the action of cell cycle inhibitors p21^{WAF1} and p27^{Kip1}, while at the same time increasing the activity of cell cycle promoters like cyclins D1 and D3. Furthermore, Akt stimulates cell metabolism and growth through activation of enzymes involved in glycolysis, glyconeogenesis, glycogen synthesis, and protein synthesis. Phospholipase C- γ , another **▶SH2 domain** – containing protein, hydrolyzes phosphatidyl 4', 5' bisphosphate (PIP₂) to diacylglycerol (DAG) and inositol-triphosphate (IP₃), once recruited to the plasma membrane following receptor activation. IP₃, a second messenger, binds to receptors on the membrane of the endoplasmic reticulum, resulting in the release of calcium, which in turn activates Ca²⁺ dependent protein kinases (e.g., the tyrosine kinase Pyk-2) and phosphatases (e.g., calcineurin). On the other hand, DAG binds to PKC, a serine/threonine kinase, thereby mediating its translocation to the membrane and its activation. Concomitant with the activation of signaling pathways, multiple mechanisms are set in motion to regulate and eventually attenuate signaling by activated growth factor receptors [5]. As in the case of EGFR and other RTKs, receptor internalization coupled to degradation is considered the most immediate and effective process that attenuates signaling by removing receptors from cell surface and targeting them for degradation in lysosomes. Phosphorylated receptors are tagged by conjugation of a 76 amino acid long molecule called ubiquitin; tagging is performed by a three-step enzymatic pathway culminating in a ubiquitin ligase called Cbl, which recognizes both the substrate receptor and the ubiquitin donor, an E2 ubiquitin-conjugating enzyme. A series of ubiquitin-binding endocytic adaptors subsequently recognize the ubiquitylated receptor and target it to regions of the plasma membrane called **▶clathrin-coated pits**. The latter invaginate to form a clathrin-coated neck that progressively moves inward, eventually generating a coated vesicle. This tiny vesicle shuttles to a vesicular compartment called the early endosome. Through acidification of the endosome's lumen, some ligand–receptor complexes are dissociated, leading to recycling to the plasma membrane, while other complexes remain intact. The latter are handed to the subsequent sorting compartment, the multivesicular body, where hydrolases are accumulated. It is in this compartment and the subsequent one, the lysosome, where degradation of the receptor is completed. Receptor activation also causes the recruitment of a number of other attenuators, such as **▶tyrosine phosphatases**, like the density-enhanced phosphatase-1 (DEP1), which dephosphorylates ErbB-1 as well as other RTKs, GTPase activating enzymes (e.g., GAP, which activates Ras GTPase activity) and other negative regulators. These proteins inhibit the signaling

cascade at multiple levels, thus realizing negative feedback loops, which help tuning and regulating the effect of growth factors.

Examples of Growth-Factor Families

EGF-Like Factors and Neuregulins

The EGF family includes EGF, transforming growth factor α , amphiregulin, epiregulin, epigen, betacellulin, HB-EGF, and neuregulins 1–4. All factors share a motif of 45–60 amino acids, including six cysteines, and their receptors belong to the ErbB family. These factors play an essential role in the development of epithelial organs, the heart, and the nervous system, as well as in tissue regeneration after injury and in wound healing. EGF-like growth factors are also involved in many kinds of cell-to-cell interactions in the adult body. For example, Neuregulin-1 is a key point in a complex signaling between neurons and glial cells, which stimulates and controls myelination. Neuregulin-1 also modulates expression and function of several neurotransmitter receptors, and altered neuregulin signaling is thought to have a role in many neurologic pathologies, like schizophrenia, multiple sclerosis, and peripheral neuropathies. Aberrantly expressed forms of ErbB proteins and their ligands are found in numerous tumors of epithelial and neuronal origins, and their presence often correlates with high tumor aggressiveness and reduced patient survival.

Fibroblast Growth Factors

Four receptors with a variety of **▶splice variants** bind more than 20 types of fibroblast growth factors (FGFs). Many FGFs require glycosaminoglycan heparin sulfate for receptor binding. FGFs play a critical role in the patterning of the embryo and in organogenesis, especially in bone development. Many genetic disorders like Chondroplasia, Apert syndrome, Pfeiffer syndrome, and Jackson–Weiss syndrome are associated with non-lethal mutations in one of the FGF receptors. FGF receptor activation has been shown to promote migration, proliferation, and differentiation of endothelial and other cells and has been implicated in angiogenesis.

Platelet-Derived Growth Factors

Disulfide-linked homologous subunits designated A, B, C, and D constitute platelet-derived growth factors (PDGFs), dimeric ligands that bind and activate two receptors, PDGFR- α and PDGFR- β . PDGF-A and PDGFR- α are expressed early while PDGF-B and PDGFR- β are expressed late during embryogenesis. Ablation of either the receptors or the ligands in mice results in embryonic or perinatal lethality with defects in alveologenesis, formation of the glomeruli in the kidney, cardiovascular disorders, and hematological abnormalities. PDGFs are potent mitogens for connective tissues and effective chemotactic factors for

inflammatory cells responsible for tissue repair and wound healing. Autocrine loops involving PDGFs have been implicated in sarcomas and gliomas, and they function in atherosclerosis, cardiac hypertrophy leading to heart failure, fibrosis of visceral organs, rheumatoid arthritis, glomerulonephritis, and proliferative vitreoretinopathy. In contrast, reduced PDGF signaling may contribute to the inability to heal chronic wounds such as diabetic ulcers.

Hepatocyte Growth Factors

The scatter factor (Hepatocyte Growth Factor; HGF) binds to Met, a single transmembrane protein with an extracellular α subunit and a membrane-spanning β subunit, whereas the macrophage-stimulating protein (MSP) binds to a related receptor called Ron. The ligands are heterodimers of A and B subunits linked by a disulfide bond. The A subunit contains one hairpin loop homologous to the plasminogen activation peptide, four **kringle domains** and a triple loop cysteine-rich motif. The B subunit is homologous to a serine protease, but it has no enzymatic activity. HGF has been implicated in liver development, conversion of mesenchyme to epithelium in organogenesis of the kidney, ovary and testes, myoblast migration, axon sprouting, and bone development. HGF expression is upregulated in response to injury in liver and kidney, where it helps regulating the process of wound healing and avoiding excessive fibrosis. Germ line and somatic mutations in Met were observed in patients with papillary renal carcinomas. Overexpression of Met has been implicated in myeloid malignancies and in carcinomas of the breast and bladder.

Vascular Endothelial Growth Factors

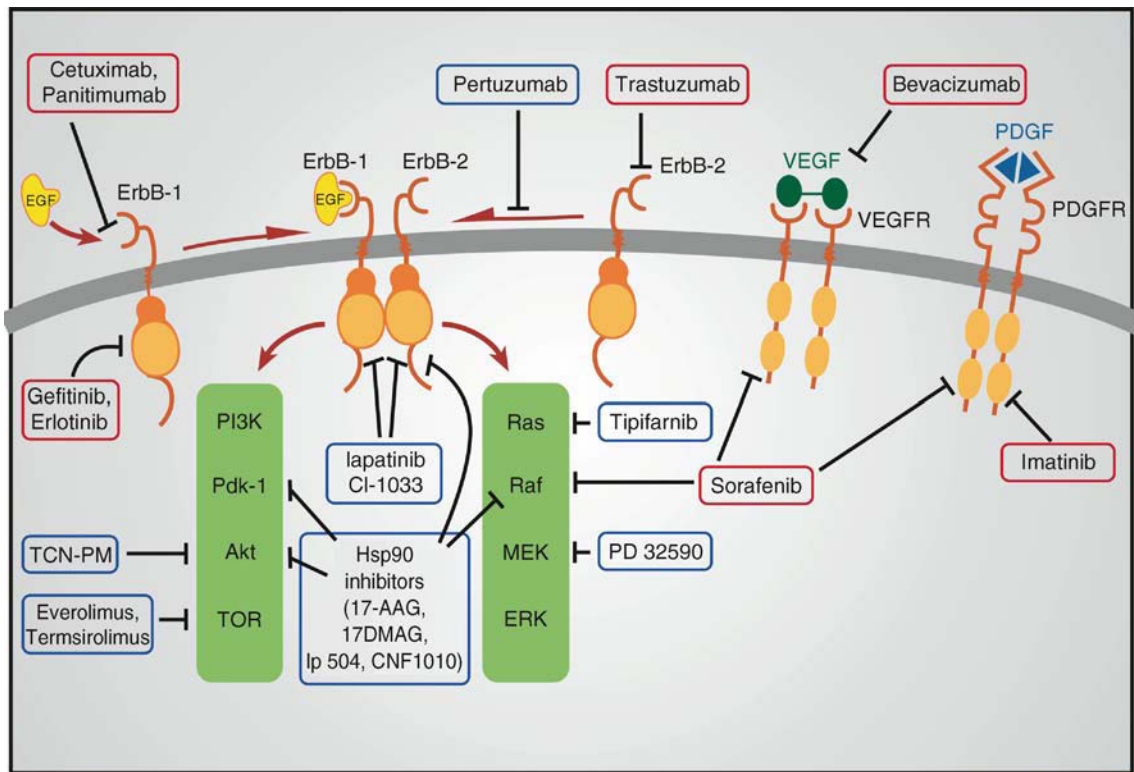
Three tyrosine kinase receptors called VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4), as well as Neuropilins (NRP) 1 and 2, are the players at the receptor level. Vascular endothelial growth factor (VEGF; also called vascular permeability factor, VPF) and its **splice variants**, as well as placental growth factor and its isoforms, serve as ligands. Activation by VEGF and semaphorin ligands results in the heterodimerization of VEGFR-2 with neuropilins. Heparin has been shown to play an important role in the signaling by this family. VEGF is predominantly an endothelial cell growth factor and is a prominent factor in the genesis and maintenance of the vasculature. VEGF and other factors are critical for physiological processes requiring angiogenesis, such as ovulation and menstruation. VEGF is induced by hypoxia and is expressed in tumors where the core is hypoxic. Secreted VEGF stimulates endothelial cells in the vicinity of solid tumors, and promotes the vascularization of the tumor and consequent tumor growth. VEGFs may also

play a role in chronic inflammatory diseases and in diabetic retinopathy, conditions characterized by excessive angiogenesis.

Clinical Applications

Several of the most formidable human diseases are characterized by aberrant signaling of growth factors and their cognate receptors, and thus these are constantly being pursued as targets for pharmacological intervention (Fig. 2). The ability of growth factors to stimulate cell proliferation and survival, for example, may help enhancing tissue healing and reduce organ damage in diseases of different origins. Recombinant forms of growth factors are currently being tested for potential usefulness in a variety of clinical settings, such as hepatitis, cirrhosis, renal fibrosis, multiple sclerosis, amyotrophic lateral sclerosis, and myocardial ischemia. Recombinant PDGF, for example, is currently employed as a drug (becaplermin) to facilitate the healing of diabetic ulcers. Growth factors may also be used for the production of artificial tissues, for example, for skin replacements and bone and connective tissue reconstruction. In contrast, blocking the actions of specific growth factors can help reduce uncontrolled cell proliferation, a characteristic of cancer (and also of diabetic retinopathy and vascular stenosis). Drugs in clinical use include small molecule **tyrosine kinase inhibitors** (TKIs) and antireceptor or antiligand monoclonal antibodies (mAbs). TKIs penetrate the plasma membrane and interact with the ATP-binding site of the receptor, thus preventing kinase activation. The ErbB-1 kinase inhibitors gefitinib and erlotinib, for instance, are both approved for a subset of Non-Small Cell Lung Cancer (NSCLC) patients. Another example is the broad-spectrum inhibitor, sorafenib, targeting both VEGFR and PDGFR, as well as the Raf kinase, which was approved for the treatment of renal cancer.

In contrast to TKIs, antireceptor mAbs bind to the extracellular domain of the receptor and exert inhibition of cell proliferation by several mechanisms. Trastuzumab, which targets ErbB-2 and is approved for the treatment of metastasizing breast cancer, is thought to stimulate an immune response against the tumor and to induce receptor internalization. Examples of other mAbs are cetuximab (approved for colorectal and head and neck cancers) and panitumumab (approved for colorectal cancer), which are directed against ErbB-1 and may act also by inhibiting ligand binding. Pertuzumab, currently in clinical trials, is an antibody which targets ErbB-2 and inhibits receptor dimerization. Some of the mAbs are directed against the ligands, rather than the receptors, an example of which is bevacizumab that targets VEGF and inhibits angiogenesis. This mAb was approved for the treatment of NSCLC and colorectal cancer; it is also being tested for many ocular



Growth Factors. Figure 2 Drugs blocking growth factor induced pathways of signal transduction. The signaling cascades set in motion by growth factors can be blocked by pharmacological interventions at different levels. Monoclonal antibodies can specifically bind to growth factors (e.g., bevacizumab) or to the extracellular domain of their cognate receptors (e.g., cetuximab). Tyrosine kinase inhibitors, on the other hand, are small molecules that traverse the plasma membrane and block kinase-domain activation. Some of them are specific to certain RTKs, like gefitinib and erlotinib (which are ErbB-1 blockers) and others inhibit the activity of multiple kinases (e.g., lapatinib or sorafenib). Several drugs block enzymes downstream of the receptors, such as drugs targeting individual effectors of the MAPK pathway or the PI3K/Akt pathway. An additional site for pharmacological intervention is the chaperone protein Hsp90, which is essential for the stability of many components of various signaling cascades, for example, ErbB-2, Raf, Pdk-1, and Akt. Inhibition of Hsp90 enhances degradation of its client proteins, leading to down regulation of the respective signals. Note that along with PDGF-receptor, imatinib inhibits also Bcr-Abl and c-Kit, the receptor for Stem Cell Factor. Red boxes indicate drugs that have already been approved for clinical use.

pathologies characterized by excessive angiogenesis, like diabetic retinopathy or macular degeneration.

Drugs that inhibit the signaling cascade downstream of the receptor at different levels (e.g., inhibitors of the MAPK or the PI3K/Akt pathways) have also been developed and are in various phases of clinical trials. ► **Chaperone** proteins like Hsp90, which are essential for the stability and function of many receptors, effectors, and transcription factors, can also be targeted by specific inhibitors (e.g., 17-AAG, 17-DMGA), thus enhancing degradation of the client proteins.

The successful clinical application of RTK-based therapeutics in the last 8 years instigates further research to generate the next generation drugs. Thus, antisense RNAs or dominant-negative receptor mutants are being tested for their ability to reduce receptor expression.

Intracellular single-chain mAbs are supposed to prevent membrane localization of the receptors. Antireceptor mAbs conjugated to radionuclides, or to prodrugs, are tested as well, and DNA vaccines may induce an active immune response against RTK overexpressing tumors.

► Cytokines

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Growth Hormone

Growth hormone (GH) is synthesized in the anterior pituitary. It belongs to the rather heterogeneous family of ►[cytokines](#). Secreted GH is a mixture of polypeptides, the main polypeptide consisting of 191 amino acids. The receptor for GH is widely distributed. Like other members of the cytokine receptor family, it possesses one transmembrane domain. A single GH molecule binds to two receptor molecules and causes their dimerization. The newly formed dimer provides a binding site for a member of the Janus kinase (JAK) family (►[JAK-STAT pathway](#)). Clinically, recombinant human GH is used for the treatment of GH deficiency. GH deficiency in children leads to short stature. GH deficiency in adults has been associated with changes in fat distribution, increases in circulating lipids, decreased muscle mass and increased mortality from cardiovascular causes.

Growth Hormone Release-inhibiting Factor

Synonyms

Somatostatin

►[Somatostatin](#)

GSK3

Glycogen Synthase Kinase 3.

►[Insulin Receptor](#)

GTPase Activating Proteins (GAPs)

GTPase activating proteins (GAPs) stimulate the intrinsic GTP hydrolysis of GTPases.

►[Small GTPases](#)

GTPases

►[Small GTPases](#)

►[Heterotrimeric G-Proteins](#)

Guanine Nucleotide Dissociation Inhibitors (GDIs)

Guanine nucleotide dissociation inhibitors (GDIs) bind to small GTPases and inhibit the dissociation and thus the exchange of the bound nucleotide.

►[Small GTPases](#)

Guanine Nucleotide Exchange Factors (GEFs)

Guanine nucleotide exchange factors (GEFs) are proteins which catalyse the release of nucleotide bound to small GTPases.

►[Small GTPases](#)

Guanylyl Cyclase

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Synonyms

Guanylate cyclase; Guanyl cyclase

Definition

Guanylyl cyclases (GC) are a family of enzymes (EC 4.6.1.2) that catalyse the formation of the second messenger cyclic GMP (cGMP) from guanosine triphosphate (GTP). GCs are subdivided in soluble GCs and GCs that are membrane-bound and linked to a receptor. Activation occurs by nitric oxide (NO) and peptide hormones, respectively [1, 2].

Basic Characteristics

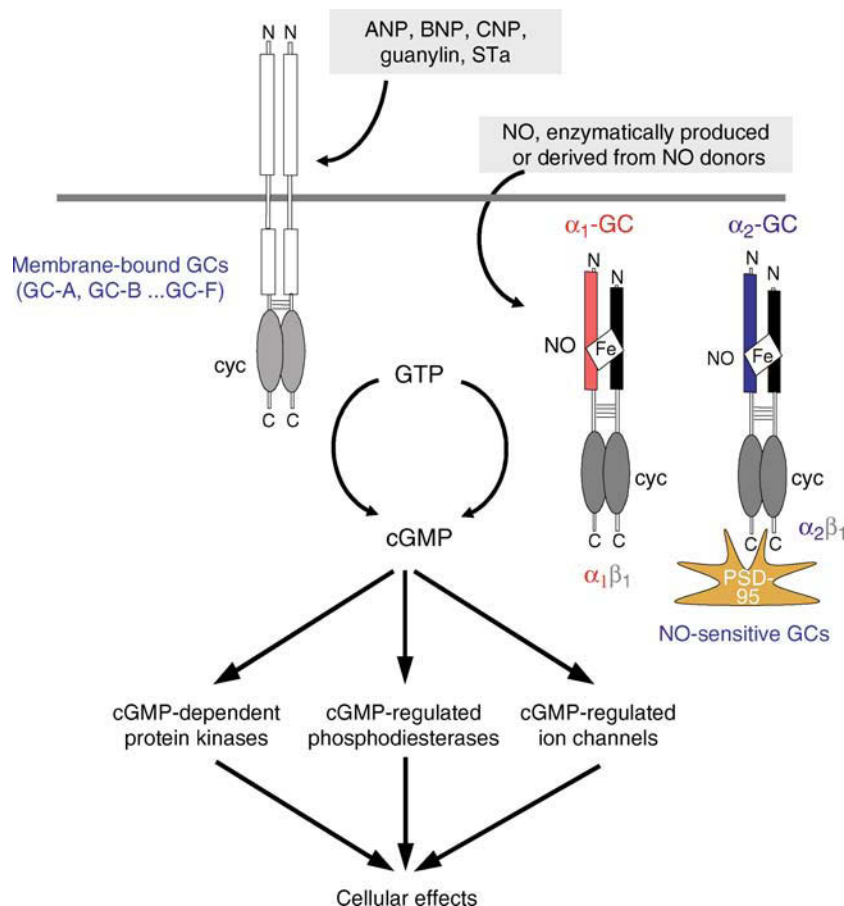
Activation of GCs leads to an increase of the intracellular messenger molecule cGMP. cGMP-signalling is mediated by three different groups of cGMP effector molecules: cGMP-activated protein kinases, cGMP-regulated **phosphodiesterases** and cGMP-gated ion channels, see Fig. 1. The cGMP increases are terminated by cGMP-degrading phosphodiesterases that reduce cGMP levels. cGMP plays a role in relaxation of smooth muscle, inhibition of platelet aggregation and in retinal phototransduction. It also participates in signal transduction within the nervous system. Moreover,

cGMP is involved in regulation of the water and electrolyte household as well as in bone metabolism. According to their structural features and their regulation, GCs can be divided into NO-stimulated and receptor-linked enzymes.

NO-Stimulated GC

NO-sensitive GC represents the most important effector enzyme for the signalling molecule NO, which is synthesised by **NO synthases** in a Ca^{2+} -dependent manner. NO-sensitive GC contains a prosthetic **heme** group, acting as the acceptor site for NO. Formation of the NO-heme complex leads to a conformational change, resulting in an increase of up to 200-fold in catalytic activity of the enzyme [1]. The organic nitrates (see below) commonly used in the therapy of coronary heart disease exert their effects via the stimulation of this enzyme.

NO-sensitive GC consists of two subunits, α and β . So far two isoforms of the enzyme have been identified; the ubiquitous $\alpha_1\beta_1$ isoform (α_1 -GC) and the



Guanylyl Cyclase. Figure 1 cGMP-signalling. Shown are the two groups of cGMP-forming guanylyl cyclases (receptor-linked membrane-bound GCs and NO-sensitive GCs) and the three effector proteins, which mediate the cGMP effects (see text for further explanation). ANP, A-type natriuretic peptide; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; Sta, heat-stable enterotoxin of *Escherichia coli*; PSD-95, post synaptic density protein 95.

less broadly distributed $\alpha_2\beta_1$ isoform (α_2 -GC). The N-terminal regions of the subunits are responsible for heme binding and heme coordination, whereas the cyclase catalytic domains are located in the C-terminal regions. The cyclase catalytic domain is conserved in the membrane-bound guanylyl cyclases as well as in the adenylyl cyclases (see below). Both isoforms show indistinguishable regulatory properties but may differ in subcellular distribution as the α_2 -GC isoform is able to interact with **▶PDZ domains** and has been shown to be associated with the postsynaptic adapter protein PDS95.

NO-sensitive GCs occur in relatively high concentration in vascular smooth muscle cells and platelets as well as in lung, kidney and brain. The NO-induced increase in cGMP causes smooth muscle relaxation and inhibition of platelet aggregation. Aside from the cardiovascular system, the NO/cGMP cascade has an important function in the nervous system, where it is thought to participate in synaptic plasticity, i.e. the use-dependent change efficiency of synaptic transmission. Knockout mice deficient in the β_1 or either one of the α subunits of NO-sensitive GC have been generated, and their phenotypes support and expand our current understanding of NO/cGMP signalling. The β_1 -deficient mice which are completely devoid of NO-sensitive GC, show a greatly reduced life expectancy and die of fatal gastrointestinal symptoms. In these mice, NO-induced relaxation is totally abolished as is NO-induced inhibition of platelet aggregation. The mice show a pronounced increase in blood pressure underlining the important role of NO in blood pressure regulation. The α_1 - or α_2 -subunit-deficient mice lack the respective isoform and therefore allow to study the role of the GC isoforms [3]. In concerns of tissues distribution, the isoforms occur in brain in similar amounts; in all other tissues tested, α_1 -GC represents the major isoform. In aortic smooth muscle cells, α_2 -GC, representing only 6% of the total GC content in WT, is sufficient to induce complete relaxation. The results show that in smooth muscle cells, α_2 -GC can functionally substitute for α_1 -GC and that a minor cGMP increase induces relaxation. Studies on synaptic plasticity in the visual cortex revealed an involvement of both GC isoforms in long-term potentiation (LTP) suggesting the occurrence of two NO/cGMP-mediated signals that have to work in concert for the expression of LTP.

Besides NO, other sGC-activating substances have been reported: Carbon monoxide (CO) is known to bind to heme groups with high affinity but has been shown to activate the enzyme only marginally (three- to fivefold). The compound YC-1 ([3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole]) is a prototype of a new class of so-called NO-sensitisers. YC-1 causes a tenfold activation of NO-sensitive GC. Pharmacologically more interesting, YC-1 increases GC's sensitivity towards NO and CO suggesting potential beneficial effects of

YC-1 by increasing the responsiveness of NO-sensitive GC towards physiologically occurring NO. Besides increasing cGMP by GC stimulation, YC-1 was shown to inhibit phosphodiesterases thereby preventing cGMP degradation and further enhancing cGMP levels in intact cells. Thus, YC-1 may represent a new class of drugs that are of potential use in the treatment of cardiovascular diseases. YC-1-related compounds with a higher affinity for NO-sensitive GC have been developed and their therapeutic benefits are currently under investigation. In addition to the NO-sensitisers, NO-independent GC activators (BAY 58-2667, HMR1766) have been identified. The observation that higher effects were observed under heme removing conditions or with the heme free enzyme led to the assumption that these activators stimulate GC by replacing and mimicking the activated NO-heme conformation. The high potency of these NO- and heme-independent activators in vivo led to the postulate of a highly responsive oxidised state of NO-sensitive GC occurring under pathophysiological situations such as oxidative stress. The assumed redox-regulation of NO-sensitive GC awaits further experimental conformation.

Receptor-linked GC

Membrane-bound GCs belong to the group of receptor-linked enzymes with one membrane-spanning region [2]. Although all of these GCs share a conserved intracellular catalytic domain, they differ in their extracellular ligand-binding domains and are activated by different peptide hormones. The guanylyl cyclase A (GC-A) isoform acts as the receptor for the **▶natriuretic peptides** ANP and BNP, two primarily cardiac hormones that are involved in the regulation of blood pressure as well as in water and electrolyte household. ANP- and BNP-induced increases in cGMP levels mediate physiological effects such as smooth muscle relaxation, modulation of endothelial permeability, inhibition of aldosterone secretion in the adrenal cortex and salt and water excretion in the kidney. During chronic pressure or volume overload, the increased ANP and BNP levels exert local antiproliferative (ANP) and antifibrotic (BNP) effects in cardiac cells. A second ANP receptor, containing only a very short intracellular C-terminal region and without any GC activity, has also been identified. As intracellular signalling of this ANP receptor has not been detected, this protein was suggested to function as a "clearance receptor", removing excess ANP from the circulating blood. Another GC isoform, GC-B, displays the highest affinity for the C-type natriuretic peptide (CNP). CNP is mainly produced by vascular endothelial cells and exhibits local and autocrine/paracrine functions. Yet, occurrence of the GC-A and -B isoforms and the natriuretic peptides is not limited to the cardiovascular system; they may also play a role in the central nervous and other systems.

Further GC isoforms are GC-C, GC-D, GC-E and GC-F. Of these GC isoforms, GC-C is stimulated by the peptide hormone guanylin that occurs mainly in the intestine. GC-C is also activated by the heat stable enterotoxin of *Escherichia coli*, which pathophysiologically, can cause severe diarrhoea. Thus, GC-C and its ligand are probably involved in regulating the salt and water balance in the intestine. The other receptor-linked GC isoforms are restricted to sensory cells. GC-D is only expressed in olfactory neurons; GC-E and GC-F are exclusively found in the retina. Regulation of these GC isoforms by proteins that interact with the intracellular cGMP-forming domain has been demonstrated. Since no ligand to the N-terminal domain of such isoforms has been identified to date, it is not clear whether cGMP-forming activity is controlled by the receptor domain at all.

Drugs

Drugs Acting on Soluble GC

Clinically, the ►organic nitrates glyceryl trinitrate, isosorbide dinitrate and isosorbide mononitrate are mainly used in the treatment of coronary heart disease. They exert their main therapeutic effect by activating sGC via NO [4]. None of the nitrates release NO spontaneously, instead they undergo a complex enzymatic bioactivation that either yields NO or bioactive S-nitrosothiols. Enzyme(s) and cofactors required for this biotransformation have not been clearly identified, yet it appears that the activity of certain enzyme(s) and cofactors can vary within different regions of the vascular system which may cause, or may contribute, to the observed differences in NO sensitivity: Since nitrate-induced vasodilation is more pronounced in veins than in arteries, the organic nitrates cause marked venorelaxation and reduce central venous pressure. In turn, the preload and the cardiac work decrease, resulting in a relief of angina pectoris symptoms. Treatments with organic nitrates that reduce cardiac preload are also used in patients with heart failure. However, direct coronary dilation or redistribution of the blood flow to ischemic regions of the myocardium remains controversial.

In general, nitrates are either used to treat or to prevent acute episodes of angina, or they are applied to provide long-term prophylaxis against episodes of angina in patients with frequent angina attacks. For the appropriate application of organic nitrates, pharmacokinetic and pharmaceutical aspects have to be taken into account. A hepatic high capacity organic nitrate reductase rapidly inactivates organic nitrates by effectively removing nitrate groups. The bioavailability of the traditional organic nitrate glyceryl trinitrate is therefore very low, and for the immediate treatment of angina, the sublingual application of glyceryl trinitrate is preferred. This way the first pass effect is circumvented and a therapeutic blood level of glyceryl

trinitrate is rapidly achieved. The nitrate can be efficiently absorbed and exert its antianginal effect within minutes. However, because the drug's duration of effect is very short (15–30 min), sublingually applied glyceryl trinitrate is not suitable for maintenance therapy. In such cases, the sublingual application of isosorbide dinitrate, which is similar to glyceryl trinitrate, is advised. In comparison, isosorbide dinitrate has a slightly delayed onset of activity but its duration of effect (2 h) is more sustained. For a drug effect that lasts even longer, nitrates such as sustained-release preparations of nitroglycerin, isosorbide dinitrate or isosorbide mononitrate are administered orally at sufficient dosage to provide effective plasma levels after first-pass-degradation. Other options to administer nitroglycerin include transdermal and buccal absorption from slow release preparations. As an active metabolite of isosorbide dinitrate, isosorbide mononitrate is available for clinical use and has a bioavailability of 100%.

The major problem of nitrate-based prophylaxis of angina is the loss of drug efficacy. The continuous application of nitrates for more than a few hours leads to the development of nitrate tolerance. Although the precise mechanisms of this tolerance phenomenon are unknown, it is conceivable that tolerance occurs at the level of the metabolising enzymes and/or the NO-sensitive GC. Moreover, an increase in the production of NO-scavenging superoxide ion and counter-regulatory mechanisms are likely to contribute to the development of tolerance. However, since the marked attenuation of the nitrate effect is rapidly reversible upon discontinuation of the drug, any tolerance development can be controlled and is achieved by allowing a "nitrate-free" period of about 8 h (usually at night) within 24 h.

An option for patients who develop nocturnal angina is molsidomine, another NO-releasing compound that is believed not to induce tolerance. Molsidomine features a similar pharmacological profile as the organic nitrates. As a pro-drug, it is bioactivated in the liver and yields SIN-1 that decomposes, enzyme-independently, in a two-step reaction. In the first step, SIN-1 undergoes a base-catalysed ring opening to form SIN-1A. This in turn yields NO and the stable metabolite SIN-1C. As the onset of action of molsidomine is comparatively slow, it is not used to treat acute cases of angina. Furthermore, due to its putative carcinogenic effect, molsidomine should only be considered when treatment with organic nitrates is not sufficient, for example in the "nitrate-free" interval.

The acute adverse effects of the organic nitrates as well as molsidomine are directly related to their therapeutic vasodilation as they can cause orthostatic hypotension, tachycardia and throbbing headache.

Apart from the substances mentioned above, there is one other NO-containing compound, sodium nitroprusside (SNP), which effectively reduces ventricular

preload and afterload. This powerful vasodilator has to be administered parenterally and is used in intensive care units to deal with hypertensive emergencies that do not respond to other blood pressure lowering agents. In the presence of reducing agents such as glutathione, SNP spontaneously releases NO concomitantly with cyanide. Its most serious adverse effects are therefore related to the accumulation of cyanide.

In low doses, inhaled NO may have a beneficial therapeutic effect, since NO in the inspired air leads to pulmonary vasodilation. In persistent pulmonary hypertension of the newborn, NO inhalation has already been used with some success. NO inhalation as the treatment for acute respiratory distress syndrome, however, has been disappointing. Only transient improvements of oxygenation were detected and the outcome of placebo-controlled trials did not show any improvement

Drugs Acting on Receptor-linked GC

In theory, one could utilise GC-A ligands to lower blood pressure and to reduce blood volume as they increase the excretion of water and salt. Nesiritide, human recombinant BNP, is the first member of this new class of drugs approved for the initial intravenous treatment of acutely decompensated congestive heart failure. Whether nesiritide can be a valuable addition to the standard therapy of decompensated heart failure remains to be demonstrated.

Besides attempts to substitute natriuretic hormones with the recombinant form of BNP, there is a pharmacological approach to elevate the concentration of natriuretic peptides by inhibiting their degradation by the neutral endopeptidase. Of special interest are dual-function inhibitors that do not only block the neutral natriuretic peptide-degrading endopeptidase but also the angiotensin-converting enzyme, thereby decreasing the level of angiotensin II. The best characterised one

of these also called “vasopeptidase inhibitors”, omapatrilat, was shown to lower blood pressure in clinical trials, however, because of the occurrence of angioedema omapatrilat has not been approved yet.

- NO Synthases
- Smooth Muscle Tone Regulation
- Phosphodiesterases
- Adenylyl Cyclases

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Gyrase

Gyrase is another term for bacterial topoisomerase II. The enzyme consists of two A and two B subunits and is responsible for the negative supercoiling of the bacterial DNA. Negative supercoiling makes the bacterial DNA more compact and also more readily accessible to enzymes that cause duplication and transcription of the DNA to RNA.

- Quinolones

Haemostasis

Haemostasis is the mechanism activated after damage to the blood vessel wall that ensures that blood loss is restricted. Blood platelets are activated and adhere to elements on the damaged luminal surface of the vessel, eventually forming a platelet plug that stops the leakage of blood. Fibrinolytic mechanisms later produce lysis of the platelet mass when repair of the vessel has occurred.

- ▶ Coagulation/Thrombosis
- ▶ Antiplatelet Drugs

Half-life/Elimination Half-life ($t_{1/2}$ or $t_{1/2\beta}$)

Half-life is the time taken to decrease the concentration of a drug to one-half its original value. There may be several phases in the elimination, and the most common is the so-called beta-phase. Alpha-phase is a distribution phase and gamma-phase is the terminal phase when the drug is finally leaving the tissues.

- ▶ Pharmacokinetics

Hamartoma Syndromes

Hamartoma syndromes are characterized by the appearance of (usually) benign tumours, consisting of large but well differentiated cells growing in a disorganized mass. Although benign, these masses, often referred to as tubers, can create serious medical problems if they develop in the brain or near essential organs.

- ▶ TOR Signalling

Haplotype

A combination of alleles that are located at closely linked loci and tend to be inherited together.

- ▶ Pharmacogenomics
- ▶ Pharmacogenetics

Hashish

- ▶ Cannabis
- ▶ Endocannabinoids

HCN

Hyperpolarisation-activated and cyclic nucleotide-gated-channel.

- ▶ Cyclic Nucleotide-regulated Cation Channels

HCV

Hepatitis C virus

- ▶ Antiviral Drugs
- ▶ Interferons

HDL

- ▶ High-Density Lipoproteins

Heat Shock Protein (HSP)

Synonyms

HSP

Definition

Eukaryotes respond to heat shock and other forms of environmental stress by inducing synthesis of heat-shock proteins (HSP). HSPs act, e.g., as chaperonins, a subclass of ►chaperones with ATPase activity, maintaining the correct 3-D structure of large protein complexes. For example, the hsp90 proteins are a group of HSPs with an average molecular weight of 90 Kd. The precise function of hsp90 is unclear. The protein is associated with steroid hormone receptors, tyrosine kinases, eIF2alpha kinase, actin and tubulin.

- Glucocorticoids
- Protein Trafficking and Quality Control
- Chaperones

Helicases

Group of evolutionary highly conserved enzymes involved in structural modulation of nucleic acids. Specific for either DNA or RNA they unwind double-stranded regions by dissociating paired purin-pyrimidin bases. A helicase domain is essential for enzymatic activity that is dependent on energy generated from nucleoside-triphosphate hydrolysis. RNA helicases are important during transcription, splicing, translation, and degradation of eukaryotic RNA.

Helicobacter Pylori

Helicobacter pylori is a spiral shaped bacterium that lives in the stomach and duodenum (the section of intestine just below the stomach). Most bacteria cannot live in the stomach since the environment is too acidic to survive. However, *Helicobacter pylori* has large amounts of urease, which converts urea to ammonia and carbon dioxide. Ammonia is used to maintain the periplasmic and cytoplasmic pH of the bacterium at neutrality, so this bacterium can survive. *Helicobacter pylori* causes peptic

ulcers, gastritis and cancer of the stomach. Peptic ulcers and gastritis are successfully treated with antibiotics effective against *Helicobacter pylori*.

- Proton Pump Inhibitors and Acid Pump Antagonists

Helix Bundle

A helix bundle is a protein composed of a series of rod-like helical domains linked by flexible segments and inserted into a membrane to form a cluster of helices roughly parallel to one another and perpendicular to the plane of the membrane.

Helix-loop-helix Motif

A helix-loop-helix motif is a DNA-binding motif, related to the leucine-zipper. A helix-loop-helix motif consists of a short α helix, connected by a loop to a second, longer α helix. The loop is flexible and allows one helix to fold back and pack against the other. The helix-loop-helix structure binds not only DNA but also the helix-loop-helix motif of a second helix-loop-helix protein forming either a homodimer or a heterodimer.

Helper T Cells

- Immune Defense

Hemangioblast

Hemangioblasts are the bipotential precursor cell population from which hematopoietic and angioblastic cells arise.

- Angiogenesis and Vascular Morphogenesis

Hematopoiesis

Hematopoiesis is the formation and development of blood cells.

►Hematopoietic Growth Factors

Hematopoietic Growth Factors

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Synonyms

Colony-stimulating factors; Hematopoietic colony-stimulating factors

Definition

Hematopoietic (blood) cells transport oxygen and carbon dioxide, contribute to host immunity, and facilitate blood clotting [1]. A complex, interrelated, and multistep process, called ►**hematopoiesis**, controls the production as well as the development of specific marrow cells from immature precursor cells to functional mature blood cells. This well-regulated process also allows for replacement of cells lost through daily physiologic activities. The proliferation of precursor cells, the maturation of these into mature cells, and the survival of hematopoietic cells require the presence of specific growth factors.

All mature blood cells arise from primitive hematopoietic cells in the bone marrow, the pluripotent stem cells. Approximately 0.1% of the nucleated cells of the bone marrow are pluripotent stem cells and approximately 5% of these cells may be actively cycling at any one time. The stem cell pool maintains itself through a process of asymmetrical cell division: when a stem cell divides, one daughter cell remains a stem cell and the other becomes a committed colony-forming cell (CFC). The proliferation and differentiation of CFCs are controlled by hematopoietic growth factors. The hematopoietic growth factors stimulate cell division, differentiation and maturation, and convert the dividing cells into a population of terminally differentiated functional cells.

Ten types of mature blood cells have been identified; each one is derived from primitive hematopoietic stem cells in the bone marrow: erythrocytes (red blood cells);

platelets (thrombocytes); granulocytes (neutrophils, eosinophils, and basophils); monocytes and macrophages; tissue mast cells; B and T lymphocytes; and plasma cells (Fig. 1).

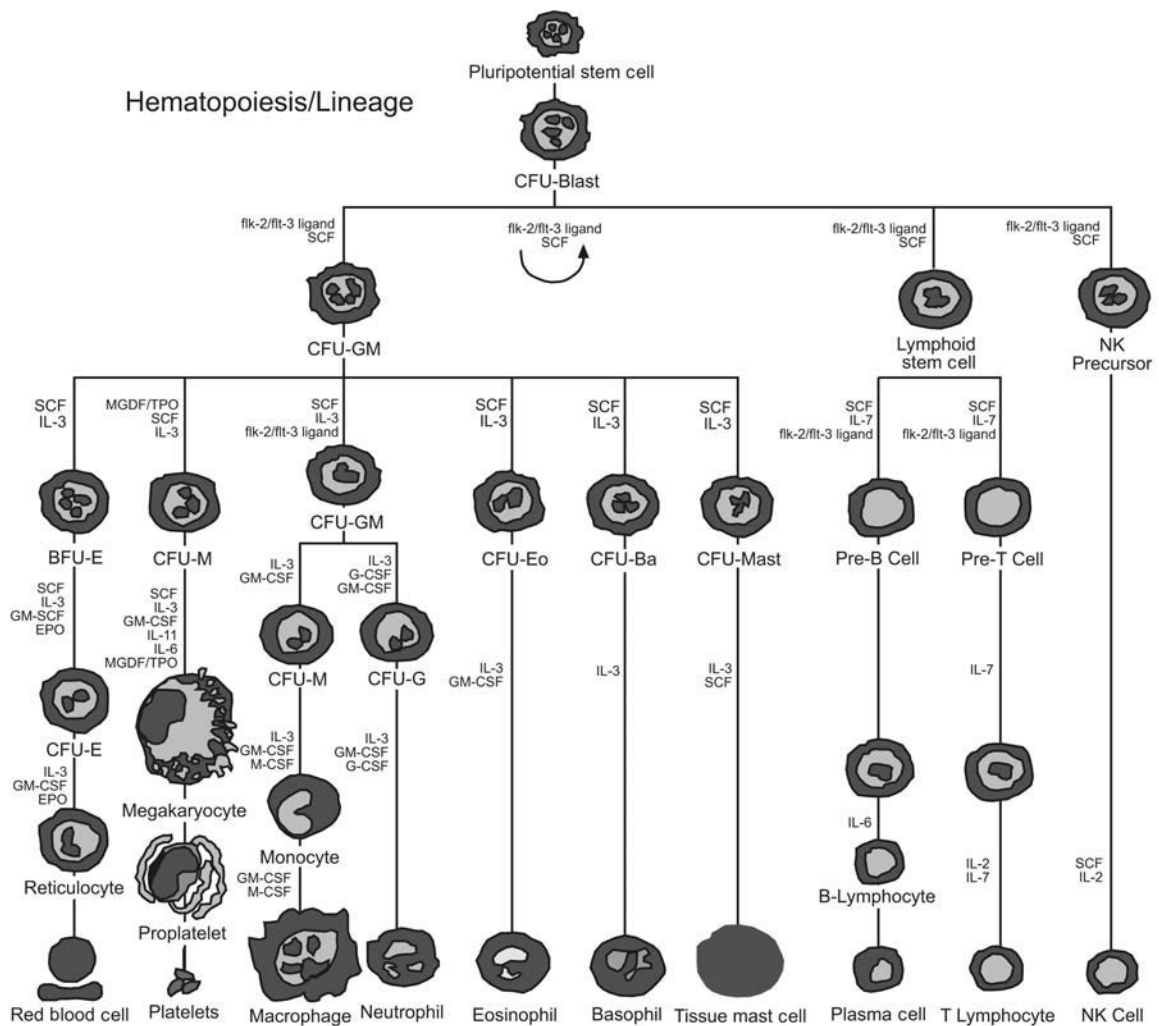
Mechanism of Action

Hematopoiesis is mediated by a series of growth factors that act individually and in various combinations involving complex feedback mechanisms to stimulate the proliferation, differentiation, and function of hematopoietic cells. The growth factors generally are glycoproteins that act through specific receptors found on the cell membrane surface of appropriate cells. The recombinant forms of hematopoietic growth factors are not always glycoproteins. The carbohydrate content varies for the growth factors and influences the mode of production for the recombinant forms and the molecular weight, but not necessarily the function of the growth factor.

More than 20 hematopoietic growth factors have been identified. The chemical properties of these growth factors have been characterized and the gene that encodes for the factor identified and cloned. Several hematopoietic growth factors are commercially available as recombinant human forms, and they have utility in clinical practice. These factors include; the recombinant forms of two myeloid hematopoietic growth factors, granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF); erythropoietin (EPO), the red cell factor; stem cell factor (SCF), an early-acting hematopoietic growth factor; and the platelet factors, thrombopoietin (TPO) and interleukin-11 (IL-11). T lymphocytes, monocytes/macrophages, fibroblasts, and endothelial cells are the important cellular sources of most hematopoietic growth factors, excluding EPO and TPO. EPO is produced primarily by the adult kidney and TPO is produced in the liver and kidney.

G-CSF (recombinant products: filgrastim, lenograstim, pegfilgrastim) maintains neutrophil production during steady-state conditions and increases production of neutrophils during acute situations such as infections [2]. Recombinant human G-CSF (rHuG-CSF) reduces neutrophil maturation time from 5 days to 1 day, leading to the rapid release of mature neutrophils from the bone marrow into the blood. The rHuG-CSF also increases the circulating half-life of neutrophils and enhances ►**chemotaxis** and ►**superoxide** production. Pegfilgrastim is a sustained-duration formulation of rHuG-CSF that has been developed by covalent attachment of a ►**polyethylene glycol** molecule to the filgrastim molecule [3].

G-CSF (recombinant products: molgramostim, sargramostim) is locally active and remains at the site of infection to localize and activate neutrophils [4]. Like G-CSF, GM-CSF stimulates the proliferation,



Hematopoietic Growth Factors. Figure 1 Schema of hematopoiesis, including some of the growth factors that influence the production of blood cells.

differentiation, and activation of mature neutrophils; and enhances superoxide production, ►phagocytosis, and intracellular killing. GM-CSF, unlike G-CSF, stimulates the proliferation, differentiation, and activation of mature monocytes/macrophages.

Erythropoietic factors (recombinant products: epoetin α , epoetin β , darbepoetin α) increase red blood cell numbers by causing committed erythroid progenitor cells to proliferate and differentiate into normoblasts, nucleated precursor cells in the erythropoietic lineage [5]. Tissue ►hypoxia resulting from anemia induces the kidney to increase its production of EPO by a magnitude of 10-fold or more. Patients with chronic renal failure are unable to produce adequate levels of endogenous EPO because of loss of renal function and must receive rHuEPO to maintain red blood cell counts. Darbepoetin α is another erythropoietic factor that has

an extended half-life due to its increased number of ►sialic acid-containing carbohydrate molecules. The newest erythropoietic factor is a third-generation molecule called CERA (continuous erythropoietin receptor activator) that incorporates a large polymer chain to produce a molecule with a half-life that is considerably longer than that of epoetin α or darbepoetin α [6].

SCF (recombinant product: ancestim) is an early-acting hematopoietic growth factor that stimulates the proliferation of primitive hematopoietic and nonhematopoietic cells [7]. In vitro, SCF has minimal effect on hematopoietic and nonhematopoietic progenitor cells, but it synergistically increases the activity of other hematopoietic growth factors, such as G-CSF, GM-CSF, and EPO. SCF stimulates the generation of ►dendritic cells in vitro and ►mast cells in vivo.

Thrombopoietic factors (no recombinant TPO product in clinical use at this time; IL-11 [recombinant product: oprelvekin] has marketing approval) stimulate the production of megakaryocyte precursors, megakaryocytes, and platelets [8]. Interleukin-11 has many effects on multiple tissues, and can interact with IL-3, TPO, and SCF. AMG 531, a recombinant peptibody in that binds to the thrombopoietin receptor Mpl and stimulates the production of platelets, is in phase 1 and 2 studies and has been shown to safely increase platelet counts in patients with immune thrombocytopenic purpura [9].

Clinical Use (Including Side Effects)

The use of recombinant hematopoietic growth factors (i.e., the commercially available forms of the native products) has been evaluated in many disorders affecting all types of blood cells. Recombinant human hematopoietic growth factors are identified as “rHu.” Not all uses discussed have received regulatory approval in all countries.

The myeloid growth factors rHuG-CSF and rHuGM-CSF have been tested and are used for the treatment of many neutrophil disorders, particularly neutropenia caused by ►myelosuppressive chemotherapy and other drugs, bone marrow transplantation, severe chronic neutropenia, leukemia, and AIDS. Neutropenia is a serious side effect of chemotherapy. Patients with neutropenia have a severely impaired ability to fight infections, and if not treated, neutropenia can be fatal. Peripheral blood progenitor cell transplantation is similar in principle to the more commonly recognized bone marrow transplantation, but collection of peripheral blood progenitor cells is less invasive than collection of bone marrow. rHuEPO is used to treat anemia, the condition of low number of red blood cells. Patients who are anemic often experience fatigue, headaches, shortness of breath, chest pain, and depression. Severe anemia can result in congestive heart failure. Anemia can be caused by myelosuppressive chemotherapy, kidney failure, and chronic diseases such as cancer. Another complication of chemotherapy is ►thrombocytopenia, an inadequate number of platelets, requiring transfusion. Thrombocytopenia can be caused by exposure to certain drugs and radiation, and can occur as the result of inherited diseases. Patients with thrombocytopenia bruise easily and may have serious internal bleeding that can cause death. rHuIL-11 acts on bone marrow to produce platelets.

When hematopoietic growth factors are used clinically, they can be associated with adverse effects. Very often patients who require hematopoietic growth factors are quite ill with their disease (i.e., cancer or kidney failure) or from their treatment (i.e., chemotherapy) and it is difficult to determine if a recombinant growth factor is responsible for a given side effect. Both rHuG-CSF and rHuGM-CSF are associated with mild-to-moderate

bone pain. It is possible that rHuGM-CSF can also be associated with fever and allergic-like reactions. In general, rHuEPO (as well as darbepoetin α) is well tolerated, but some patients experience flu-like symptoms, hypertension, or headaches. The most common event associated with rHuSCF is injection-site reactions (i.e., ►edema or ►urticaria). Administration of rHuIL-11 is associated with several toxicities, including fluid retention, anemia, and cardiac arrhythmias.

The recombinant hematopoietic growth factors have had a significant impact on the treatment of cancer, including prevention of serious infections and anemia.

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Heme

Heme ($C_{34}H_{32}O_4N_4Fe$) represents an iron–porphyrin complex that has a protoporphyrin nucleus. Many important proteins contain heme as a prosthetic group. Hemoglobin is the quantitatively most important hemoprotein. Others are cytochromes (present in the mitochondria and the endoplasmic reticulum), catalase and peroxidase (that react with hydrogen peroxide), soluble guanylyl cyclase (that converts guanosine triphosphate, GTP, to the signaling molecule 3',5'-cyclic GMP) and NO synthases.

►Guanylyl Cyclase

Hemodialysis

Renal replacement by an artificial device providing intermittent clearance of plasma based on the physical principle of diffusion.

► Pharmacokinetics

Hemofiltration

Renal replacement by an artificial device providing continuous filtration of plasma based on the physical principle of convection.

► Pharmacokinetics

Hemostasis

► Coagulation/Thrombosis

Hemozoin

Hemozoin, also known as malaria pigment, is, in terms of its chemical composition, identical to β -hematin. Hemozoin is formed as a crystallization product of heme under the acidic conditions present in the food vacuole of malarial parasites. In the crystal, the heme molecules are linked into dimers through reciprocal iron-carboxylate bonds to one of the propionate side chains of each porphyrin. The dimers form chains linked by hydrogen bonds.

► Antiprotozoal Drugs

Heparin Sulfate Proteoglycans

Heparin sulfate proteoglycans (HSPGs) are heavily glycosylated proteins that are part of the extracellular matrix. Interaction with HSPGs help to stabilize and localize extracellular Wnts.

► Wnt Signaling

Hepatic Lipase

Synonyms

HL

Definition

Endothelial-anchored enzyme in liver primarily responsible for hydrolysis of triglycerides and phospholipids in Intermediate Density Lipoproteins (IDL) and High Density Lipoproteins (HDL).

► Lipoprotein Metabolism

Hepatitis

Hepatitis is acute or chronic inflammation of the liver, which is frequently caused by infection with hepatotropic viruses. Several forms of viral hepatitis (A, B, C, D, E) are known, which result from infection with viruses belonging to separate virus families, differing in their genomic organization, replication strategies, morphology and modes of transmission.

► Viral Proteases

► Interferons

► Antiviral Drugs

Hepatitis C

Hepatitis C Virus (HCV) is the causative agent of hepatitis C, identified in 1989. HCV is a positive-strand RNA virus of the Flaviviridae family, known to infect human liver. Infection may be symptomless but may cause severe liver damage after several years. It is estimated that 1–3% of the world population are infected with HCV. The main route of transmission is parenteral with high incidence among intravenous drug users. Other risk factors are tattooing and needle stick injuries, whereas sexual transmission appears to be infrequent. About 20% of HCV cases cannot be associated with risk factors (sporadic infections) but correlate with low socio-economic background and enhanced contact with risk groups. Prior to the identification of the virus, blood transfusions, blood products or renal dialysis could lead to infection, due to unchecked contamination with HCV. Nowadays, reliable diagnostic assays are used to monitor the presence

of HCV in blood or blood products and have diminished the chance of infection to less than 1 in a million transfused units.

- ▶ Viral Proteases
- ▶ Interferons
- ▶ Antiviral Drugs

Heptahelical Domain

Heptahelical domains are protein modules found in all known G-protein coupled receptors, made up of seven transmembrane helices interconnected by three extra and three intracellular loops. For most G-protein coupled receptors activated by small ligands, the binding site is located in a cavity formed by transmembrane domains 3, 5, 6 and 7.

- ▶ G-protein-coupled Receptors

Heptahelical Receptors

- ▶ G-protein-coupled Receptors

HERG-channels

HERG-channels are voltage-gated K^+ channels which belong to the ether-à-go-go (eag) family of K_v -channels. HERG stands for human eag related gene. Mutations in the human gene are associated with the long QT-syndrome LQT2. Together with MiRP1 (MinK-related peptide 1), HERG constitutes the cardiac rapid delayed rectifier K^+ channel (IK_R) which is responsible for the repolarization of cardiac action potential and therefore governs the action potential duration. Many drugs ranging from erythromycin to sotalol may block HERG-channels. Certain mutations in HERG-channels are associated with drug-induced arrhythmia.

- ▶ K^+ Channels
- ▶ Voltage-gated K^+ Channels

Heterologous Desensitization

Heterologous desensitization is a form of desensitization which does not require agonist binding of the receptor. Second messenger dependent kinases such as protein kinase A (PKA) and protein kinase C (PKC) are involved in this form of receptor desensitization. Heterologous desensitization simply depends on the overall kinase activity which is regulated by many different stimuli.

- ▶ Tolerance and Desensitization

Heterologous Expression System

Heterologous expression systems comprise prokaryotic organisms (e.g., *E. coli*) and eukaryotic cells (e.g., yeast, HEK293, *Xenopus* oocytes), which are used to functionally express foreign genes or cDNAs.

Heterotrimeric G-Proteins

Synonyms

G-proteins; Heterotrimeric GTP-binding proteins

Definition

Heterotrimeric G-proteins are part of a widely used transmembrane signaling system consisting of G-protein coupled receptors or binding proteins, (GPCRs), heterotrimeric G-proteins and a variety of effectors (enzymes or ion channels). Activation of GPCRs by extracellular ligands leads, via the activation of G-proteins, to the regulation of effectors. Heterotrimeric G-proteins consist of α -, β -, and γ -subunits. In order to convey a signal from an activated receptor to an effector, the heterotrimeric G-protein undergoes an activation–inactivation-cycle, which allows it to function as a regulatory molecular switch. In the basal state, the β/γ -complex as well as the GTP-bound α -subunit are associated, and this complex is recognized by an appropriate activated receptor. Interaction with the receptor results in the dissociation of GDP from the α -subunit and its replacement by GTP. Binding of GTP induces a conformational change, which results in the

dissociation of the α -subunit and the β/γ -complex. Both, the α -subunit and the β/γ -complex, are now able to regulate effectors. The reassociation of heterotrimeric G-protein is induced by the hydrolysis of GTP to GDP. Two ►bacterial toxins have been found to specifically interfere with the G-protein activation–inactivation cycle, ►pertussis toxin blocks the interaction of the activated receptor with several members of the $G_{i/o}$ -family of G-proteins whereas cholera toxin leads to the constitutive activation of some G-proteins including G_s . The inactivation of G-proteins is enhanced by various effector molecules as well as by a group of proteins called ►“regulators of G-protein signaling” (RGS-proteins), which act as GTPase activators. There are four main subfamilies of G-proteins which are classified according to the identity of their α -subunits. The G_s -family mediates the stimulatory regulation of ►adenylyl cyclases, whereas the $G_{i/o}$ -family mediates the inhibitory regulation of adenylyl cyclases. $G_{i/o}$ -family members are also involved in the stimulatory regulation of K^+ -channels (►GIRK) and the inhibitory regulation of some ►voltage-dependent Ca^{2+} -channels. The $G_{q/11}$ is involved in the stimulatory regulation of β -isoforms of ►phospholipase C. The $G_{12/13}$ -family couples receptors to the activation of the ►small GTPase RhoA.

- G-protein-coupled Receptors
- Transmembrane Signalling
- Table appendix: Heterotrimeric G-proteins

Hidden Markov Model

A Hidden Markov Model (HMM) is a general probabilistic model for sequences of symbols. In a Markov chain, the probability of each symbol depends only on the preceding one. Hidden Markov models are widely used in bioinformatics, most notably to replace sequence profile in the calculation of sequence alignments.

- Bioinformatics

High-density Lipoprotein (HDL)

Lipoprotein fraction with apolipoprotein A-I as structural protein. HDL is believed to carry cholesterol away from the blood vessels and back to the liver. High

plasma concentrations are associated with a reduced risk of heart disease. HDL is also some times called “the good cholesterol.”

- Lipid Transfer Proteins
- Lipoprotein Metabolism
- Sterol Transporters
- HMG-CoA-Reductase Inhibitors (Statins)

High-throughput Screening

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Definition

High-throughput screening (HTS) is the term used to describe the portion of the ►drug discovery process in which ►compound libraries are tested for an effect in an ►assay directed against a molecular ►target or biological mechanism. Although there is no precise definition of high-throughput per se, any process capable of performing 10,000 or more tests per day is generally considered a high-throughput screen. Achieving this screening rate often involves the use of robotics and automation to perform some or all steps in the assay. Therefore, the fundamental components comprising HTS are an assay, a compound collection, and some automated methods for carrying out the screen. Compounds that are identified as active by HTS are called hits and may be the starting points for further chemical optimization. A target is a protein or protein assembly whose function is believed to be important for promoting health or treating disease. An assay is used to measure the effect of the test compound on the target. There are many different assay formats that are compatible with HTS. However, in order to be suitable for HTS, an assay should be sensitive, informative, reliable, and simple to implement (i.e., relatively few steps). Assay performance can be measured statistically using commonly accepted standards. HTS is an important component of modern drug discovery and an area of active research and development in the pharmaceutical and biotechnology industries.

Description

Types of Targets

Any protein whose function is believed to be clinically relevant and suitable for modulation by a drug can be considered a drug target. Targets may be grouped

according to their functional class. Historically, many mammalian targets have been cell surface molecules such as receptors, transporters, or ion channels. More recently, intracellular targets such as kinases and nuclear receptors have received increased attention [1]. The targets in many infectious diseases are the genes essential for microbial growth or viral replication. It is estimated that current drugs target between 300 and 350 different molecular targets [2]. Estimates regarding the number of novel, therapeutically relevant targets that remain to be discovered as potential drug targets vary widely.

Types of Assays

Broadly defined, assays fall into two major categories: biochemical and cell based. Biochemical assays are based on isolated protein preparations, while cell-based assays utilize intact cells. Usually, cell-based assays rely on recombinant mammalian cell lines in which the target of interest is heterologously expressed. There are many different types of target-based assays ranging in complexity and information content. Among the simplest assays are ligand-binding assays that measure displacement of a radiolabeled ligand from a purified protein preparation by a test compound. These assays are best applied when the target has a well-defined binding site. G-protein coupled receptors (GPCRs) represent such a target class. For membrane-bound targets such as GPCRs, these binding assays can be carried out in membrane preparations. Binding assays, while simple, do not provide information on whether a test compound affects the function of a target.

Functional assays provide information as to whether a test compound activates, inhibits, or otherwise modulates the target. Functional biochemical assays can be performed on purified proteins such as enzymes (e.g., kinase protease). These assays typically utilize substrates to indicate the turnover rate of the enzyme. The most complex assays are cell-based functional assays. These assays are favored when it is necessary to measure the effect of a compound on target function in a cellular environment. For example, many ion channels are not ligand regulated and/or require multi-subunit assemblies to function. Therefore, the most practical way to screen these targets is to measure their activity in an intact cell. Measurements of ion flux, ion concentration, or membrane potential are all viable methods to screening these targets. Cell-based functional assays can be developed for most targets and are particularly useful when one is screening for a particular type of modulator. For example, in addition to detecting target activation or inhibition, these assays can identify allosteric modulators of target activity.

Not all cell-based functional assays are directed against a specific molecular target. Indeed many drugs

have been identified based on an observed effect of a compound on a cellular phenotype, such as shape, viability, or growth. An example of this is cyclosporin, a natural product initially identified for its antimicrobial effects and later found to inhibit T-cell proliferation. Such assays can be considered “phenotypic” in the sense that one is assaying for a change in some measurable cell parameter. The advantage of these types of screens is that they do not introduce a target-centric bias to drug discovery. A challenge for these assays is the difficulty in identifying the molecular mechanism of compound action, and the consequence of developing drugs without full understanding their mode of action. An alternative use of phenotypic assays is to screen for novel targets [3].

Assay Formats

While it is beyond the scope of this article to describe all the assay formats used in HTS, a general description of the types of readouts is pertinent. In general, HTS assays are either endpoint or kinetic assays. Endpoint assays take only a single read from a well (possibly with a pre-read before compound or reagent addition) and allow more flexibility for automation while kinetic assays require more precise processes and timings associated with multiple reads from the same well. Compounds may be preincubated or added acutely to the assay. Because HTS is an automated process, assay formats with minimal steps are preferred. The most favored assays are so-called “homogeneous” assays that require only addition steps and lack exchange or separation steps. Most assays utilize either radioactive, absorbent (colored), fluorescent, or luminescent reagents to determine the activity of the test compound in the assay. Optical detection methods for both biochemical and cell-based assays have improved in quality, sophistication, and ease of use in recent years and have contributed greatly to advances in the field. Because of the importance of the assay in the screening process, competitive advantage may be afforded to those who are proficient in the design and implementation of screening assays. The reader is referred to reviews for more information [4, 5].

Screening Formats

HTS is usually carried out in multiwell plates and the industry has settled on certain standard formats. One key standard is that most screening assays are carried out in 96 (8 × 12) or 384 (16 × 24) well plates with a standard footprint. This standard is important because most automation is optimized for use with these plates and attendant assay volumes in the 100 μ L range. Furthermore, source plates containing test compound are typically stored in a similar configuration, allowing for more efficient transfer of test compound from the source plate to the assay plate. Various types of plates are available to match different assay formats, including

opaque plates for scintillation counting and clear plates for absorbance or fluorescence assays. For cell-based assays, plates are available with special surface coatings or treatments to render them suitable for incubation of cells. The industry is experimenting with miniaturization beyond 384 (e.g., 864, 1536, and 3456 well plates and assay volumes in the 1–10 μL range). Although there are promising examples of success in this area, miniaturized formats are not in widespread use as of 2002. This is due in part to the fact that the higher density formats require novel instrumentation and automation processes and represent a high barrier to entry. The industry is also experimenting with fundamentally different approaches such as flow-based, format-free, or panning methods. These methods face similar or greater barriers to entry as high-density plates. As technologies improve and costs decrease one would expect to see increased use of both high-density plate formats and, perhaps, completely novel HTS formats.

Compound Libraries

The quantity and quality of compound libraries are key considerations and drivers for HTS. The compound libraries of large pharmaceutical and screening companies can exceed 1 million samples, creating the need for significant infrastructure and technologies to complement HTS activities. There has been significant retrospective analysis of biological and chemical descriptors associated with “drug likeness” [6, 7] and many companies are attempting to fill chemical “space” with smaller, more precisely focused compound collections. Thus, many companies are reassessing the need for ever-larger libraries, with a prevailing trend toward quality rather than quantity. Nevertheless, drug discovery is still an inexact science and screening libraries in most companies still contain at least 100,000 samples. Libraries are synthesized and stored as either individual samples or as combinations. Individual samples offer the advantage of generally being of higher purity and allow for more rapid follow up. However, they are costly to generate and this method is not amenable to rapid exploratory chemistry. In the past decade, parallel synthesis and other methods have led to the notion of “combinatorial” chemistry in which sets of compounds are generated simultaneously [8]. These reactions generate multiple variants on a chemical theme and these are often stored as mixtures to be tested. Because both methods offer some advantage, many screening libraries are composed of compounds generated by both methods. Historically, most compounds were synthesized in house, making each library unique. More recently chemistry companies have begun commercializing libraries, so the prospect exists that many companies are screening similar, if not identical compounds against identical targets.

Automation and the Screening Process

With an assay and screening library in hand, automation and instrumentation complete the HTS process. An HTS lab typically consists of at least one automated system capable of moving plates from one task station to another. This is often accomplished by means of a robotic arm with substations arranged in either a linear or a radial fashion. A minimum suite of stations consists of an automated pipettor to add assay reagents or compounds, incubators (or racks) to store assay and/or compound plates, and a plate reader to detect the assay results. Additional stations may remove plate lids, change the orientation of plates, or read barcodes to keep track of plates. The steps associated with different assays can vary significantly depending on whether compounds are preincubated and/or whether the readout is endpoint or kinetic. Because different assays require different processes and timings, the efficiency of the system is maximized by software.

Measuring HTS Output: Data Quality and Validated Hits

If an assay has been properly designed and executed against an appropriate target and compound collection, the result should be quality data that identifies chemical starting points for further chemical optimization and drug development. Data quality (i.e., assay and screen performance) can be measured by objective statistical criteria based on assay statistical separation of positive and negative controls [9], but the quality and value of hits is less quantitative. Hits are scored and prioritized by biological and chemical criteria. Biological criteria include potency and selectivity against the target while chemical criteria take into account novelty, chemical properties, synthetic tractability, and scientific intuition.

Conclusion

HTS is a relatively young field, with some of the earliest references dating to the early 1990s and its impact on the efficiency of the drug discovery is yet to be fully assessed [1]. Like most new fields, the value of HTS to drug discovery is the subject of debate. Only time and disclosure by pharmaceutical companies of internal historical data will provide the metrics for long-term assessment of the method of broad screening activities. Indeed, one successful outcome of HTS would be if the generation of data sets that create a more rational basis for predicting which compounds will be appropriate for a given biological target. Thus, one role of HTS is to eventually limit the need for random screening of compound libraries. In addition, HTS methods are seeing broader applications in profiling compounds for other information relevant to drug design, including selectivity, physical properties (e.g., solubility), and in vitro metabolism. In an even broader sense, HTS has

driven and will continue to influence thinking about the role of engineering and automation at the interface of chemistry and biology. One undisputed legacy of HTS will likely be an increase in the technological sophistication of discovery research.

► Combinatorial Chemistry

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High-voltage-activated Ca^{2+} Channels

► Voltage-dependent Ca^{2+} Channels

Hill Analysis

A method for analysing saturation or competition binding curves from radioligand binding assays to determine whether the interaction between the ligand and receptor is competitive. The slopes of the Hill plot or coefficient should equal one if the ligand binds competitively. Slopes significantly greater than one may indicate

positive cooperativity; slopes less than one may indicate heterogeneity in binding or negative cooperativity.

► Drug-receptor Interaction

Hippocampus

The hippocampus, which got its name from the Greek word for seahorse, due to its form, is a nucleus in the depth of the temporal lobe. The hippocampus is important for the integration of sensory information, for spatial orientation and for memory formation. The hippocampal formation contains the ‘CA’ (cornu ammonis) regions, the dentate gyrus and the subiculum.

Hirudin

Hirudin is a polypeptide derived from the saliva of the leech *Hirudo medicinalis* that binds to the blood serine proteinase, thrombins, and thus blocks clot formation.

► Anticoagulants

► Proteinase-activated Receptors

Histamine

Neurotransmitter and biogenic amine derived from the amino acid histidine synthesized in hypothalamic tuberomammillary neurons (TMN) to maintain wakefulness, feeding rhythms, energy balance, neuroendocrine autonomic control, and memory functions; prominent immunomodulator and proinflammatory signal released from mast cells in response to allergic reactions or tissue damage.

► Histaminergic System

► Inflammation

► Allergy

► Orexins

► Emesis

Histamine and Emesis

Histamine may act as a neurotransmitter in the brain although it acts as an autacoid in the periphery. Histamine H1 receptors are present in the vestibular nucleus along with a high density of muscarinic cholinergic receptors. High densities of histamine H1 receptors are also found in the nucleus tractus solitarius and in the dorsal motor nucleus of the vagus. Intermediate levels are found in the nucleus ambiguus but are not detected in the area postrema. Histamine may also play a functional role in the vestibular apparatus since it increases firing of afferent nerves in the ampulla, an effect inhibited by histamine H1 and H2 receptor antagonists. It should be noted that not all central histamine H1 receptor antagonists are useful antiemetics. Many of those that are effective, such as diphenhydramine and promethazine also possess some ability as muscarinic receptor antagonists and this may contribute to their effectiveness. However, cyclizine is an effective antiemetic without having any marked affinity for muscarinic receptors.

► Emesis

Histaminergic System

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Definition

Histamine is a biogenic amine that is widely distributed in the body and functions as a major mediator of inflammation and allergic reactions, as a physiological regulator of gastric acid secretion in the stomach, as a neurotransmitter in the central nervous system (CNS) and may also have a role in tissue growth and repair.

Basic Characteristics

Histamine is stored within granules of mast cells in almost all tissues of the body and has a major role as a local hormone (►autocoid) in the generation of allergic and inflammatory reactions. It is found in particularly high concentrations in mast cells in the lungs, skin and gastrointestinal tract and is also present in circulating basophils. ►Allergens and antigens bind to IgE antibodies on the surface of mast cells causing the IgE

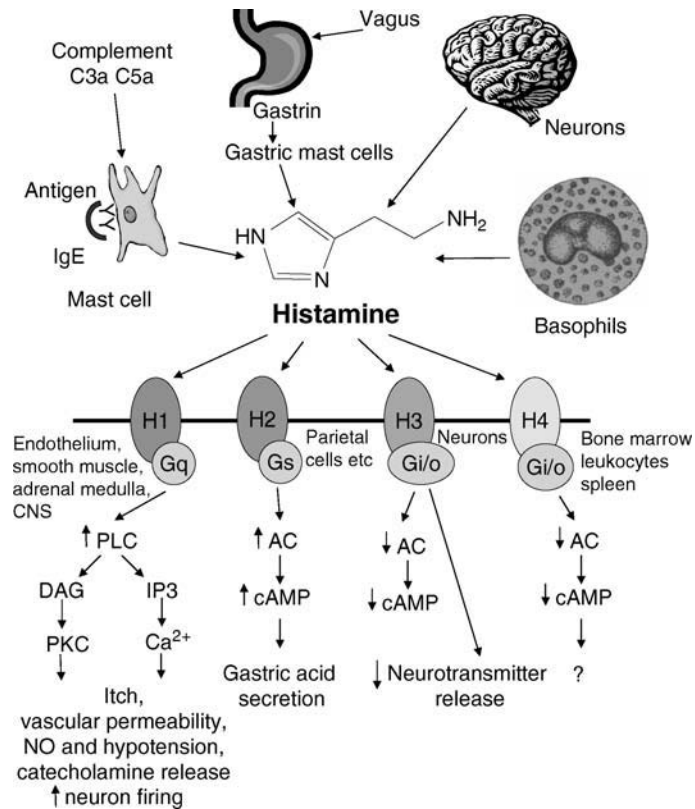
to crosslink. This conformational change stimulates the release of pre-stored histamine (degranulation) from mast cells. Direct interaction of components of the ►complement system (C3a and C5a) with specific cell surface receptors can also trigger mast cell degranulation. A number of clinically used drugs (e.g. morphine, tubocurarine) and neuropeptides (at high concentrations, e.g. substance P) can also stimulate histamine release from mast cells directly via non-receptor mechanisms. In the gastric mucosa, histamine from stomach mast cells has an important physiological role in the secretion of gastric acid. Parasympathetic nerve stimulation (acetylcholine via the vagus) and gastrin release from G cells both activate gastric mast cells, releasing histamine. All three stimuli are able to act synergistically to activate the neighbouring parietal cells to produce more gastric acid.

Although mast cells and basophils probably account for >90% of stored histamine in the body, histamine is also present in platelets, enterochromaffin-like cells, endothelial cells, and neurons. Histamine can act as a neurotransmitter in the brain. Histaminergic nerves have their cell bodies within a very small area of the brain (the magnocellular nuclei of the posterior hypothalamus) but have axons in most areas of the forebrain. There is also evidence for axons projecting into the spinal (Fig. 1) cord. Finally, there is evidence that histamine synthesis can be induced in tissues undergoing rapid tissue growth and repair. In certain neonatal tissues (e.g. liver), the rate of synthesis of this unstored diffusible histamine (termed nascent histamine) is profound and may point to a role for histamine in cell proliferation.

Histamine is synthesized from the amino acid histidine via the action of the specific enzyme histidine decarboxylase and can be metabolized by histamine-*N*-methyl transferase or diamine oxidase. Interesting, in its role as a neurotransmitter the actions of histamine are terminated by metabolism rather than re-uptake into the pre-synaptic nerve terminals.

Receptors for Histamine

Histamine binds to and activates cell surface receptors. Four such receptors for histamine have now been identified so far (H₁, H₂, H₃, H₄) and their structural sequences determined following molecular cloning. All four are members of the G-protein-coupled receptor family and mediate their functional responses by activating specific ►heterotrimeric G-proteins. H₁-receptors coupled to G_{q/11}-proteins and mediate responses primarily via the activation of phospholipase C, which hydrolyses membrane phospholipid phosphatidylinositol-4,5-bis phosphate into the intracellular second messengers inositol 1,4,5-tris phosphate (IP₃) and diacylglycerol. IP₃ is released into the cytosol and stimulates the release of Ca²⁺ ions from intracellular stores, while



Histaminergic System. Figure 1 The Histaminergic system.

diacylglycerol remains within the plasma membrane and mediates responses via the activation of protein kinase C. Histamine H₂-receptors couple to G_s-proteins and stimulate the enzyme adenylyl cyclase, which is responsible for the synthesis of the second messenger cyclic AMP. In contrast, both the H₃- and H₄-receptors couple to the G_{i/o}-family of G-proteins, which inhibit adenylyl cyclase activity, and, in the case of the H₃-receptor, also inhibit neurotransmitter release in the CNS.

H₁-Receptor

The human histamine H₁-receptor is a 487 amino acid protein that is widely distributed within the body. Histamine potently stimulates smooth muscle contraction via H₁-receptors in blood vessels, airways and in the gastrointestinal tract. In vascular endothelial cells, H₁-receptor activation increases vascular permeability and the synthesis and release of prostacyclin, platelet-activating factor, Von Willebrand factor and nitric oxide thus causing inflammation and the characteristic 'wheal' response observed in the skin. Circulating histamine in the bloodstream (from, e.g. exposure to antigens or allergens) can, via the H₁-receptor, release sufficient nitric oxide from endothelial cells to cause a profound vasodilatation and drop in blood pressure (septic and anaphylactic shock). Activation of

H₁-receptors in the adrenal medulla stimulates the release of the two catecholamines noradrenaline and adrenaline as well as enkephalins. In the heart, histamine produces negative inotropic effects via H₁-receptor stimulation, but these are normally masked by the positive effects of H₂-receptor stimulation on heart rate and force of contraction. Histamine H₁-receptors are widely distributed in human brain and highest densities are found in neocortex, hippocampus, nucleus accumbens, thalamus and posterior hypothalamus where they predominantly excite neuronal activity. Histamine H₁-receptor stimulation can also activate peripheral sensory nerve endings leading to itching and a surrounding vasodilatation ('flare') due to an axonal reflex and the consequent release of peptide neurotransmitters from collateral nerve endings.

H₂-Receptor

The histamine H₂-receptor (359 amino acids) is best known for its effect on gastric acid secretion. Histamine H₂-receptor activation, in conjunction with gastrin and acetylcholine from the vagus, potently stimulate acid secretion from parietal cells. High concentrations of histamine are also present in cardiac tissues and can stimulate positive chronotropic and inotropic effects via H₂-receptor stimulation and activation of adenylyl

cyclase. In smooth muscle, H_2 -receptor stimulation leads to relaxation and this has been observed in airway, uterine and vascular smooth muscle. In the immune system, histamine H_2 -receptors can inhibit a variety of functions. For example, H_2 -receptors on lymphocytes can inhibit antibody synthesis, T-cell proliferation and cytokine production. In the CNS, H_2 -receptor activation generally leads to inhibition of nerve cell activity; however, in hippocampal neurones they produce a block of the long-lasting after-hyperpolarization and accommodation of firing, leading to potentiation of excitatory stimuli.

H_3 - and H_4 -Receptors

The histamine H_3 -receptor (445 amino acids) was first identified as an **▶autoreceptor**, negatively regulating the synthesis and release of histamine from histaminergic neurons in the CNS. However, H_3 -receptors have been identified on the terminals of many neurons in both the CNS, where they can inhibit the release of acetylcholine, serotonin, dopamine and noradrenaline, and on peripheral neurons where they inhibit the release of sympathetic neurotransmitters in human saphenous vein, heart, bronchi and trachea. Unlike the genes for H_1 - and H_2 -receptors, the H_3 -gene contains **▶introns** (three **▶introns** and four **▶exons**) thus, multiple H_3 -receptor isoforms (**▶splice variants**) can be produced from the single H_3 -receptor gene. So far, several isoforms of the H_3 -receptor have been detected in different species including six splice variants in the human CNS and in some of these cases, the subsequent signalling functions appear to be different. As several different H_3 -splice variants exist with different signal transduction capabilities, this splicing mechanism offers a way for tightly regulating the biological actions of the H_3 -receptor in different tissues. The H_3 -receptor also appears to be expressed in a **▶constitutively active** (Receptors are thought to exist in at least two states; (1) an inactive state and (2) an active state where the receptor can bind to its G-protein and elicit a functional response. Under basal conditions, the equilibrium between the two states of the receptor is substantially in favour of the inactive state. Agonists normally bind with higher affinity to the active form of the receptor and alter the equilibrium in a manner to increase the proportion of receptors in the active state. Sometimes, however, there is sufficient active receptor present under basal conditions to produce a measurable response in the absence of agonist. In this situation the receptor is termed to have constitutive activity (inverse agonist).) form in the CNS providing a means for pharmacological interference by **▶inverse agonists** as well as agonists and **▶neutral antagonists**.

The H_4 -receptor (390 amino acids) is the most recently identified and unlike the H_3 -receptor appears to

be exclusively expressed in the periphery. The H_4 -receptor shows highest levels in bone marrow and leukocytes (mainly neutrophils and eosinophils), moderate levels in spleen and small intestine and has been detected on mast cells. It has a genomic structure consisting of two introns and three exons suggesting that splice variants may also occur.

Drugs

H_1 -Antagonists

A large number of drugs have been developed as histamine H_1 -receptor antagonists. These include mepyramine, chlorpheniramine, promethazine, triprolidine, diphenhydramine, cyclizine and cyproheptadine (although many of these are actually **▶inverse agonists**) and have proved to be very effective in the treatment of systemic and topical allergic and inflammatory disorders (hay fever, allergic rhinitis, insect bites, anaphylaxis, etc.). At therapeutic doses, many of the traditional antihistamines give rise to sedative effects because of blockade of the H_1 -receptors in the brain. More recently, a second generation of H_1 -antihistamines has been synthesized, which has poor blood-brain barrier penetration and therefore cause less central sedative effects. These include temelastine, acrivastine, astemizole, cetirizine and loratidine. Both chlorpheniramine and cetirizine exist as stereoisomers with markedly different affinities for the human histamine H_1 -receptor. In the case of cetirizine, the active levo-isomer is now available for clinical use. Many H_1 -receptor antagonists also possess marked muscarinic receptor antagonist properties (e.g. promethazine, diphenhydramine, cyclizine) and this 'side-effect' is exploited for the treatment of nausea and motion sickness. Several other classes of drugs, namely the antidepressants doxepin, amitriptyline and mianserin and the antipsychotic drug chlorpromazine, are also potent H_1 -antihistamines.

H_2 -Antagonists

The first H_2 -antagonist that had selectivity for H_2 - over H_1 -receptors was burimamide; however, this compound is now known to be a more potent H_3 -receptor antagonist. Cimetidine was developed directly from burimamide and proved to be an effective agent in the treatment of gastric and duodenal ulceration because of its ability to inhibit basal and gastrin-stimulated gastric acid secretion. A wide range of highly selective H_2 -receptor antagonists are now available and are in regular clinical use including ranitidine, titotidine, nizatidine, famotidine and mifentidine. Most H_2 -receptor antagonists penetrate poorly into the CNS, but zolantidine is an example of a selective brain-penetrating H_2 -receptor antagonist. Studies in transfected cells overexpressing the H_2 -receptor and demonstrating constitutive receptor activity have shown that cimetidine and ranitidine are both **▶inverse**

agonists at the H₂-receptors, while burimamide behaves as a ►neutral antagonist.

H₁- and H₂-Receptor Agonists

Selective agonists, like selective antagonists, are useful for scientific characterization of receptors. Although not clinically used, a number of potent agonists are available, which are able to selectively stimulate the histamine H₂-receptor. These include impromidine, arpromidine, sopromidine, dimaprit and amthamine. In the case of impromidine and apromidine, the compounds are 10–100 times more potent than histamine itself. However, while acting as agonists at the H₂-receptor, some of these compounds act as antagonists at other histamine receptors, e.g. arpromidine is a potent H₁-receptor antagonist, and impromidine a potent H₃-receptor antagonist. Selective agonists for the H₁-receptor (histaprodifen, *N*-methylhistaprodifen) have only become available recently.

Ligands for the Characterization of Histamine

H₃- and H₄-Receptors

Agonists with good selectivity for H₃-receptors (relative to H₁- and H₂-receptors) have been developed and these include R- α -methylhistamine, imetit and imnepip. H₃-receptor antagonists include thioperamide, clobenpropit, iodoproxyfan, ciproxifan and impentamine and these all have substantially lower affinity for H₁- and H₂-receptors. The histamine H₄-receptor was discovered and cloned in 2000 and it is clear that there is considerable overlap in the pharmacology of the H₃- and H₄-receptors. High affinity H₃-agonists, e.g. R- α -methylhistamine, imetit and imnepip also have H₄-agonist properties although, their ►relative potency with respect to histamine is generally lower. For example, R- α -methylhistamine is several hundred-fold less effective as an H₄-receptor agonist than as a H₃-receptor agonist. The H₃-receptor antagonist clobenpropit also binds with high affinity to the H₄-receptor, but also possesses weak H₄-agonist activity. Thioperamide is an antagonist at both the H₃- and H₄-receptors, but has 5- to 10-fold lower affinity for the H₄-receptor. Thioperamide also has inverse agonist activity at both receptors. UCL 2138 (H₃ antagonist) and JNJ 7777120 (H₄ antagonist) represent two antagonists that have good selectivity between H₃ and H₄ receptors. Perhaps the most striking aspect of the pharmacology of the new H₄-receptor, however, is the fact that the atypical antipsychotic drug clozapine has agonist activity at the H₄-receptor but no agonist or antagonist activity at the H₃-receptor.

Drugs, Which Inhibit Histamine Synthesis, Metabolism and Release

Histamine synthesis from l-histidine can be selectively inhibited by α -fluoromethylhistidine. Metabolism by

diamine oxidase is sensitive to aminoguanidine, while *N*-methylation of histamine by histamine methyltransferase can be inhibited by SKF 91488, tacrine and metoprine. Sodium cromoglicate and nedocromil are drugs that inhibit mediator release from mast cells (e.g. histamine) and are used in the prophylaxis of asthma (in children), allergic rhinitis and allergic conjunctivitis. Ketotifen is an H₁-receptor antagonist that has also been reported to inhibit mast cell degranulation.

►Inflammation

►Allergy

►Orexins

►Emesis

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Histone

Histones are small, basic proteins required to condense DNA into chromatin. They have been first described and named in 1884 by Albrecht Kossel. There are five main histones: H1, H2A, H2B, H3 and H4. An octamer of core histones H2A, H2B, H3 and H4 is located inside a nucleosome, the central building block of chromatin, with about 150 base pairs of DNA wrapped around. The basic nature of histones, mediated by the high content of lysine and arginine residues, allows a direct interaction with the acidic phosphate backbone of DNA. The fifth histone H1 is located outside at the junction between nucleosomes and is referred to as the linker histone. Besides the main histones, so-called histone variants are known, which replace core histones in certain locations like centromeres.

►Histone Acetylation

Histone Acetyl Transferases (HAT)

An enzyme activity ascribed to many coactivators, which transfers acetyl groups to lysine residues of histone tails of the nucleosomes and thereby facilitate their disruption and the opening of the chromatin.

- Histone Acetylation
- Transcriptional Regulation

Histone Acetylation

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Synonyms

Lysine acetylation of histones

Definition

► **Histone** acetylation is a reversible and covalent modification of histone proteins introduced at the ϵ -amino groups of lysine residues. Histones and DNA form a complex – ► **chromatin** – which condenses DNA and controls gene activity. Current models interpret histone acetylation as a means to regulate chromatin activity.

Basic Mechanisms

All organisms must deal with the problem of packing DNA molecules of enormous size into the limited space of their cellular nuclei. Eukaryotes solve this problem by organizing their DNA into chromatin, a complex of DNA and dedicated packing proteins, so-called histones. This strategy allows an enormous reduction of the DNA's spacial requirement, up to 10,000-fold. Chromatin consists of repetitive building blocks called ► **nucleosomes** comprising four pairs of histones (H2A, H2B, H3, and H4) with about 150 base pairs of DNA wrapped around. The nucleosome is only the first stage of packing DNA and further condensation into higher-order chromatin structures takes place in order to reach most efficient packaging. The structure of condensed higher-order chromatin is still unknown, but the nucleosomal building blocks with their histone cores are maintained as the basic structural unit [1].

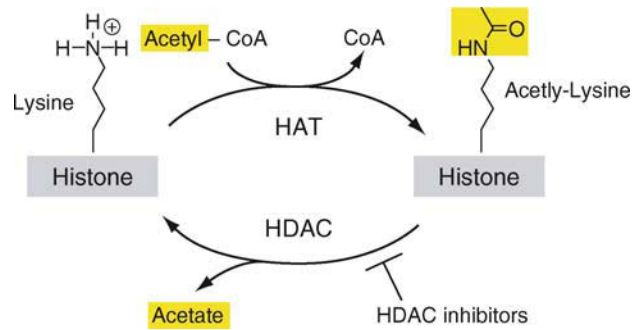
Histone proteins share distinct features including a high content of basic amino acids, lysines and arginines,

which represent up to 25% of all residues. They consist of C-terminal globular domains located inside the nucleosome, which interact with the DNA and other histones. In addition, histones possess N-terminal ► **histone tails** which appear largely unstructured based on X-ray crystallographic data and reach outside the nucleosomes. Whereas the globular domains form the scaffold around which the DNA is wrapped, the function of the histone tails is less obvious. Histone tails are not necessary for nucleosomal assembly or stability; however, their amino acid sequence is highly conserved throughout all eukaryotic organisms indicating an important biological function of some sort. Another intriguing discovery was that histone tails are subjected to intensive posttranslational acetylation of lysine residues [1, 2]. Although some histone acetylations have been discovered on the globular domains as well, the histone tails are the primary acetylation sites with about 20 positions known to date. Several experiments have shown that histone acetylation has a strong impact on gene activity. Two theories, the “charge neutralization model” and the “histone code theory” offer explanations of how histone acetylation mediated gene regulation might work.

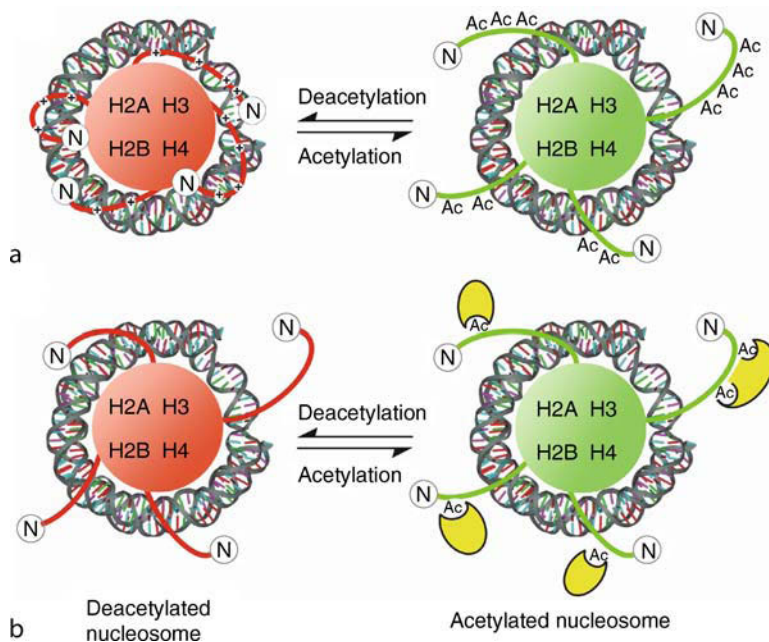
The Role of Histone Acetylation in Gene Regulation

The first model assigning a physiological function to histone acetylation is based on the chemical change inflicted onto the lysine residues upon acetylation. As illustrated in Fig. 1 histone acetylation is a chemical conversion of a lysine's amino group into an amide group. This conversion results in the loss of a positive charge, which naturally occurs under physiological conditions, due to protonation of the amino group (see Fig. 1). According to the “charge neutralization model” the charged histone's tails interact tightly with the negatively charged phosphate back-bone of the DNA (see Fig. 2a). As a result the affected DNA is inaccessible to regulatory proteins like transcription factors and the encoded genes cannot be transcribed. This inactivation is reversed by histone acetylation, which neutralizes the charge of the histone tails. The tails detach from the DNA and genes within the deacetylated chromatin regions can be transcribed (see Fig. 2a). The “charge neutralization model” is capable of explaining several aspects of chromatin activity. However, recent investigations have revealed further details which require extensions of the current model.

A model called “histone code theory” includes more aspects of chromatin regulation which have been identified. The “histone code theory” predicts that histone acetylation and other posttranslational histone modifications serve as binding sites for regulatory proteins which mediate processes like gene transcription upon recruitment (see Fig. 2b) [3]. In this context histone modifications can be understood as



Histone Acetylation. Figure 1 Histone acetylation is a posttranslational modification of lysine residues of histones. This modification is catalyzed by histone acetyl transferases (HATs), which transfer an acetyl group (yellow) from acetyl-Coenzyme A onto the ϵ -amino group of the lysine residue. Histone deacetylation is catalyzed by histone deacetylases (HDACs), which hydrolyze the lysine bound acetyl group. HDAC inhibitors like Trichostatin A (TSA) are known to inhibit the deacetylation reaction in vivo and in vitro.



Histone Acetylation. Figure 2 Current models describing how histone acetylation can regulate chromatin activity. The nucleosome consists of two pairs of histone proteins H2A, H2B, H3, and H4 with 147 base pairs of DNA wrapped around. The N-terminal tails of the histones reach outside the nucleosome and can be acetylated at certain lysine residues. (a) "Charge Neutralization model": Due to their positive charge the histone tails interact tightly with the DNA and block access to the DNA. As a result the chromatin is inactive (red). Histone acetylation neutralizes the charge and opens up the chromatin structure to become active (green). (b) "Histone code theory": Patterns of histone acetylations and other modifications serve as recruitment sites for regulators of chromatin activity (yellow), which are needed to activate chromatin.

an instrument to store instructions about how the adjacent DNA should be handled. This theory takes into account further posttranslational modifications including ►histone phosphorylation and ►histone methylation have been identified which do not necessarily change the charge of the histone tails like lysine methylation [1, 3].

In addition protein domains have been identified which bind to modified histone tails. The so-called bromodomains bind to acetylated histone tail, but have little or no affinity to unmodified tails. Further known binding domains include chromodomains and SANT domains which possess preferential binding to methylated and unmodified tails.

An important aspect of the “histone code theory” is that some modifications can be passed on during cell division. As a result histone modification patterns including histone acetylation serve as a means to store inheritable traits of an organism which are not DNA encoded. This kind of information is generally termed ►epigenetic information.

The exact role of individual histone acetylations will have to be determined in the context of other modifications and the number of lysine residues effected. However, the general importance of histone acetylation as a regulator for chromatin activity is undisputed. This leads to the intriguing possibility to develop drugs that target histone acetylation for therapeutic purposes. The primary targets for drug development are the histone acetyl transferases (HATs) and the histone deacetylases (HDACs) which introduce and remove histone acetylations [2, 3].

Histone Acetyl Transferases (HATs)

Acetylation of lysine residues is catalyzed by dedicated enzymes, the so-called histone acetyl transferases (HATs). These enzymes use the ubiquitous metabolite acetyl-CoenzymeA (Acetyl-CoA) as the acetate source (see Fig. 1). The reaction is performed in a nonrandom manner and different HATs possess preferential acetylation sites. The first HATs to be identified belong to the GCN5 family. The corresponding gene in yeast has long been known to encode a transcriptional regulator and subsequent biochemical studies have proven the HAT-activity. Further HATs have been found including CBP/p300, which is involved in cell cycle progression and cyclic-AMP dependent gene activation. CBP/p300 is known to be a transcriptional coactivator which means it does not have preferential chromatin binding sites but can be recruited to specific locations by sequence-specific DNA binding proteins like transcription factors. Upon recruitment CBP/p300 acetylates the tails of the adjacent nucleosomes and thereby opens the chromatin structure for further transcriptional initiation factors [1, 2].

HATs do not appear as sole catalytic entities and are always embedded in large multi-protein complexes, which can comprise up to 20 different proteins and are more than 2 MDa in size [1, 3].

Histone Deacetylases (HDACs)

Histone Deacetylases (HDACs) catalyze the removal of the acetyl groups from lysines (see Fig. 1). Together with the HATs they are responsible for maintaining the level of histone acetylation throughout the genome. The family of HDAC proteins has been divided into four classes based on phylogenetic analysis and sequence comparison. HDACs of the classes I and II share the same Zn^{2+} -based reaction and are evolutionary related. Class IV HDACs also possess a Zn^{2+} -based reaction

mechanism but seem to have no relation to class I and II HDACs. The final class of HDACs (class III), which are better known as Sir2 proteins or sirtuins, share neither evolutionary nor mechanistic relations with the other HDACs. They do not require Zn^{2+} for catalysis but strictly depend on the cofactor NAD^+ , which they use as the acceptor of the acetyl group. Eleven Zn^{2+} -dependent HDACs of the classes I, II, and IV named HDAC 1–11 are known in mammals. In case of the sirtuins seven homologs named SIRT 1–7 have been identified in humans [2].

Like HATs, most functional HDACs are embedded in large multifunctional protein complexes, which also contain other chromatin modifying enzymes and coregulator proteins [1].

Pharmacological Relevance

The precise balance of the acetylated and deacetylated state of histones is an important feature of gene regulation. Imbalances can be found in many human cancers and often result from alterations in HDAC and HAT activities. In this context overexpression or aberrant recruitment of HDACs by oncoproteins are common in human cancers, as well as mutations in HAT encoding genes. These findings have challenged scientists to discover and explore chemical compounds capable of modulating such aberrant HDAC/HAT activities for academic and medical purposes.

Development of HDAC inhibitors (HDACi's) have been most successful to date. The first known type of HDACi's were short-chain fatty acids like butyric acid that inhibits HDACs in the millimolar range in vivo and in vitro [2]. However, these compounds suffer from a short in vivo half-life resulting in low plasma levels. In order to overcome these shortcomings prodrugs have been developed like Pivanex, which shows promising effects in different cancer cell lines and possess better plasma stability. The best known HDACi is the natural product Trichostatin A (TSA), which inhibits HDACs in the low nanomolar range and cells treated with TSA show a significant increase in histone acetylation levels. TSA is a hydroxamic acid based HDACi which inhibits HDACs through tight coordination of the essential Zn^{2+} -ion. However, TSA is primary of academic interest due to poor pharmacokinetics [2]. The most advanced HDACi to date is Zolinza also known as SAHA or Vorinostat. This compound belongs to the class of hydroxamic acids as well and inhibits HDACs in the nanomolar range. Zolinza is the first HDACi to be approved by the US Food and Drug Administration for treatment of certain malignant diseases [2]. Besides the short-chain fatty acids and hydroxamic acids other classes of HDACi's including electrophilic ketons, amino-benzamides, and cyclic peptides are currently being developed [2].

The HDACi's mentioned above inhibit exclusively Zn^{2+} -dependent HDACs and not the NAD-dependent Sirtuins. Researcher also focuses on development of sirtuin inhibitors and first successes like the compound "sirtinol" have been reported. Finally, the development of HAT inhibitors is also pursued and to date some compounds like the peptide-based inhibitor "H3-CoA-20" or the small molecule "MB-3" are among the first molecules to show HAT inhibition [3].

►Transcriptional Regulation

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Histone Deacetylases

Synonyms

HDAC

Definition

Enzyme activity ascribed to corepressors, which is the removal of acetyl groups from lysine residues of histone tails. Thereby the assembly of nucleosomes is maintained, which leads to a dense, transcriptional inactive chromatin structure.

►Transcriptional Regulation

►Histone Acetylation

identified recently. Histone methylation seems to mediate long-term repression of transcription by recruitment of potent transcriptional repressors, such as heterochromatin protein-1 (HP-1).

►Histone Acetylation

►Transcriptional Regulation

Histone Phosphorylation

Histone phosphorylation is a common posttranslational modification found in histones, primarily on the N-terminal tails. Phosphorylation sites include serine and threonine residues, tyrosine phosphorylation has not been observed so far. Some phosphorylation events occur locally whereas others occur globally throughout all chromosomes during specific events like mitosis. Histone phosphorylation is catalyzed by kinases. Removal of the phosphoryl groups is catalyzed by phosphatases.

►Histone Acetylation

Histone Tails

Histone tails are the N-terminal regions of histones which reach outside the nucleosomes. They are not essential for the formation of nucleosomes but are required for the formation of higher-order chromatin structures. The histone tails are also known to be heavily posttranslationally modified by acetylation, phosphorylation, methylation, etc. and are important for the regulation of gene activity.

►Histone Acetylation

Histone Methylation

Histone methylation is a common posttranslational modification found in histones. Histone methylations have been identified on lysine and arginine residues. In case of lysines S-adenosyl-methionine (SAM) dependent methyl transferases catalyze the transfer of one, two or three methyl groups. Lysine methylation is reversible and lysine specific demethylases have been

HIV

The human immunodeficiency virus (HIV) is the causative agent of the acquired immunodeficiency syndrome (AIDS). HIV is a retrovirus, whose replication includes the transcription of the single-stranded RNA genome into double stranded DNA (reverse transcription) and the covalent insertion of the DNA

copy of the viral cDNA into the genome of the host cell (integration). Both steps are mediated by virus-encoded enzymes.

- ▶ Antiviral Drugs
- ▶ Viral Proteases
- ▶ Chemokine Receptors

HMG Box

The HMG (high mobility group) box is a DNA-binding domain found in several transcription factors, that can in some cases bend DNA. Some members of this protein family recognize a unique DNA sequence, whereas others bind to a common DNA conformation.

- ▶ Cadherins/Catenins

HMG CoA-Reductase

Rate-limiting enzyme in cholesterol biosynthesis; inhibition by “statins” results in reduction of plasma LDL-cholesterol levels.

- ▶ HMG-CoA-Reductase Inhibitors
- ▶ Lipoprotein Metabolism

HMG-CoA-Reductase Inhibitors

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Odense University Hospital and Department
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Synonyms

Statins

Definition

A class of important pharmacological compounds that are the most effective drugs for lowering plasma levels of low-density-lipoprotein (LDL)-cholesterol.

Mechanism of Action

HMG-CoA-reductase inhibitors (statins) inhibit the enzyme HMG-CoA-reductase that catalyzes the conversion of acetyl coenzyme A to mevalonate, which through several further biochemical steps is metabolized to cholesterol (Fig. 1). HMG-CoA-reductase is the rate-limiting enzyme for the endogenous formation of cholesterol in the human body, which takes place primarily in the liver. The reduced production of cholesterol leads to an increased formation (upregulation) of so-called ▶LDL-receptors on the surface of hepatic cells that remove cholesterol from the circulating blood. By this, the body ensures that its demand for cholesterol (cholesterol is a constituent of cell membranes, bile acids, and various hormones) is met, and subsequently plasma cholesterol is lowered. A second, although probably less important mechanism, by which statins reduce plasma cholesterol is by a reduction in hepatic synthesis of ▶very-low-density-lipoprotein (VLDL) and LDL. Statins therefore markedly reduce plasma levels of ▶atherogenic LDL-cholesterol and slightly reduce plasma ▶triglycerides carried in VLDLs and in addition slightly increase plasma levels of antiatherogenic ▶high-density-lipoprotein-cholesterol.

Furthermore, there is some evidence for pleiotrophic effects (e.g., effects on hemostasis, vascular function, anti-inflammatory effects, and stabilizing effects on ▶atherosclerotic plaques) of statins. The clinical relevance of this (and the potential difference between the various statins) is at present uncertain but subject to intense investigation.

Clinical Use

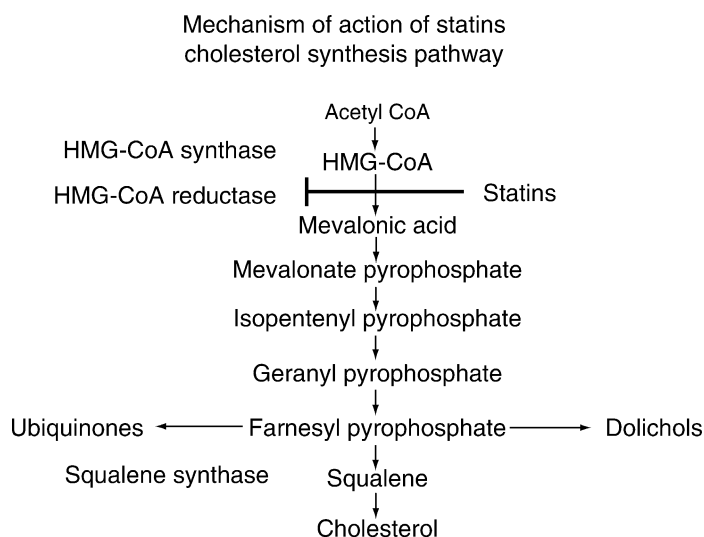
Statins are used to reduce LDL-cholesterol in subjects believed to have too high levels of cholesterol to reduce their risk of cardiovascular and in particular coronary heart disease. They are used both in patients with established vascular disease and subjects considered at high risk for cardiovascular disease, including subjects with genetic disorders of lipid and lipoprotein metabolism. Statins are by far the most commonly used and the most effective class of drugs for pharmacological lowering of plasma cholesterol. Statins were introduced as investigational drug compounds in the early 1980s and has been marketed since 1987.

Types of Statins

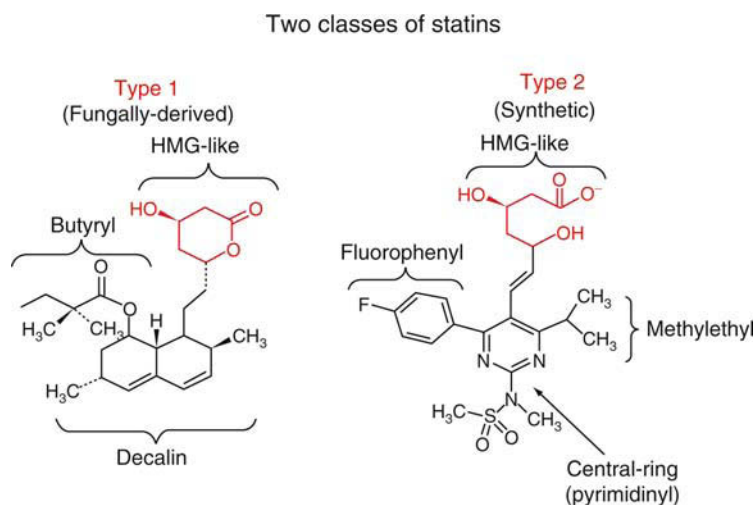
There are currently marketed four naturally derived statins (lovastatin, pravastatin, simvastatin, and rosuvastatin) and two synthetic statins (atorvastatin and fluvastatin). The structure of these statins is shown in Fig. 2.

Basic Pharmacology

The individual statins differ with respect to their pharmacological properties, but in general the clinical consequences of this are limited, but may occasionally



HMG-CoA-Reductase Inhibitors. Figure 1 Mechanism of action of statins – cholesterol synthesis pathway. The conversion of acetyl CoA to cholesterol in the liver. The step of cholesterol biosynthesis inhibited by HMG-CoA reductase inhibitors (statins) is shown.



HMG-CoA-Reductase Inhibitors. Figure 2 The chemical structure of the fungal-derived and the synthetic HMG-CoA reductase inhibitors (statins).

be of importance with respect to side effects and interactions with other pharmaceutical drugs. Atorvastatin has the longest half-life and can be given at any time of the day, while other statins are best administered in the evening (lovastatin with the evening meal), perhaps due to the fact that hepatic cholesterol biosynthesis is greatest during the night time. Despite this, all of the statins can be given in one daily dose. The statins are mainly metabolized by the liver (approximately 90%) apart from pravastatin, where 60% of the drug is excreted by the kidneys. Therapeutically, much caution should be given if patients with liver or renal diseases are treated, and if statins are used in such

patients low doses should be administered under tight control.

Statins should not be used in pregnant women. If women with child-bearing potential are treated with statins efficient contraception should be secured. Statins should at present not be used in children unless they carry a very high risk of premature vascular disease and in this case only by very experienced lipid specialists.

Effect on Plasma Lipids and Lipoproteins

Statins may reduce total cholesterol and LDL-cholesterol levels in plasma by 50–60%, reduce plasma

triglycerides by up to 30% (best effect in individuals with high triglycerides) and increase HDL-cholesterol by 5–10%. It should, however, be mentioned that responsiveness to statins differ substantially between individuals. The major effect of statins is to reduce the atherogenic LDL-cholesterol fraction and this effect is dose dependent and typically increases by 6% for each doubling of the starting (lowest) approved dose of the drug. The effect on LDL-cholesterol varies between preparations in maximal approved doses from 30 to 60% in the following order of efficacy: fluvastatin < pravastatin < lovastatin < simvastatin < atorvastatin < rosuvastatin.

The effect of statins on plasma lipids and lipoproteins is rapidly seen and fully achieved after 4–6 weeks of treatment. The effect persists unchanged during continued use for several years, but after stopping the drug, LDL-cholesterol rapidly increases to pretreatment levels. Treatment with statins is therefore usually continued indefinitely and not as a short-term cure. Finally, it is generally advisable to use the statins that have documented their efficacy in clinical trials (evidence-based medicine).

Effect on Clinical Events

Several major well-conducted clinical trials have been published where subjects (4–10,000) have been treated with a statin or matching placebo for approximately 5 years. These studies have included patients with coronary heart disease as well as patients without preexisting heart disease. Moreover, more recent trials have included patient groups with diabetes, peripheral artery disease, and stroke. The results have been remarkably similar, with a reduction in deaths from vascular disease and heart attacks, and the number of revascularizations (bypass surgery or balloon angioplasty) of roughly 30% of the subjects given statin compared to those receiving the placebo. The reduction in mortality from coronary heart disease has been achieved without any indication of adverse trends in noncoronary mortality. Moreover, the benefit has been observed in various age groups including old people, in both genders and in groups of patients with elevated plasma cholesterol as well as in patients with cholesterol values considered normal. Recent trials have been focusing on more intensive lipid lowering by comparing high dose with low-dose statin therapy. The results clearly showed clinical benefit from aggressive lowering of LDL-cholesterol in patients with cardiovascular disease.

Side Effects

Abdominal symptoms including changes in bowel function, rash, and disturbances of sleep have been reported, but in general statins are remarkably free of side effects. Thus, in the large clinical trials comprising

several thousands of patients treated for approximately 5 years, side effects and the rate of discontinuation due to suspected side effects have been very similar in individuals receiving statins and placebo.

Two types of side effects, however, need to be considered. Approximately 1% of the subjects experience an increase in liver enzymes during statin treatment, and it is generally advised that a (repeatedly found) increase in liver enzymes to more than 2–3 times above normal levels should lead to discontinuation of the statin. This side effect is asymptomatic to the patient, reversible and generally occurs shortly after institution of treatment and with high doses of the statin. Myositis which, during continued treatment may progress to **rhabdomyolysis** and acute renal failure, is a serious but very rare complication seen in less than 0.2% of patients. The risk of myositis is greatest when a statin is given together with certain other drugs including (but not exclusively) erythromycin (antibiotic), nicotinic acid and fibrates (other lipid-lowering drugs), ciclosporin (used in transplanted patients), and some drugs used for systemic treatment of fungal infections. One statin, cerivastatin, was recently (year 2001) withdrawn from the market due to an unacceptably high incidence of rhabdomyolysis, in particular when used in combination with gemfibrozil.

If side effects occur on one statin, a change to another statin may be tried under supervision, but commonly the same side effect is encountered during treatment with the new statin. Statins (in particular simvastatin and lovastatin) that are metabolized via a hepatic enzyme system (C450) may interact with drugs metabolized by the same system (e.g., diltiazem and anticoagulant drugs) increasing plasma levels of the statin and/or the simultaneously used other drug. To date, however, few patient cases of clinically relevant drug interactions have been reported with the statins.

Treatment Control

The effect of a statin is usually determined by measuring fasting plasma lipids and lipoproteins after 4–6 weeks of treatment. Liver enzymes and eventually creatine kinase (in case of myositis liver enzymes are usually also elevated) are measured simultaneously to exclude side effects related to liver and muscles. After the treatment goal has been reached, blood sampling is usually performed 1–2 times a year.

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Homologous Desensitization

Homologous desensitization is a form of desensitization which is mediated by agonist-induced activation of the same receptor. ►G-protein-coupled receptor kinases (GRKs) and arrestins are involved in this process, which leads to an uncoupling of the receptor from its G protein.

- Desensitization
- Heterologous Desensitization
- Tolerance and Desensitization

Homologous Proteins

Two proteins with related folds and related sequences are called homologous. Commonly, homologous proteins are further divided into orthologous and paralogous proteins. While orthologous proteins evolved from a common ancestral gene, paralogous proteins were created by gene duplication.

- Bioinformatics

Homologous Recombination

Homologous recombination is a form of genetic alteration that occurs when DNA duplexes align at regions of sequence similarity and new DNA molecules are formed by the breakage and joining of homologous segments.

- Transgenic Animal Models

Homology Modeling

Computationally deriving a 3D molecular structure of a given protein using a sequence overlay with a related protein of known structure.

- Molecular Modelling

Hormonal Contraceptives

- Contraceptives

Hormone Replacement Therapy (HRT)

Estrogens and progestins are diminished in menopausal or ovariectomized women. In hormone replacement therapy (HRT), these hormones are substituted to alleviate hot flushes, mood changes, sleep disorders, and osteoporosis.

- Selective Sex Steroid Receptor Modulators
- Sex Steroid Receptors: Androgen Receptors, Estrogen Receptors, Progesterone Receptor

HPA Axis

Hypothalamus-pituitary-adrenal Axis.

- Glucocorticoids
- Glucocorticoid Receptors
- Antidepressant Drugs

HRT

- Hormone Replacement Therapy

Hsp

- ▶ Heat Shock Proteins
- ▶ Chaperones

Hsp70

Hsp70 is a molecular chaperone (relative molecular mass 70 kD) found in different compartments of eucaryotic cells. Hsp70 was originally described as heat shock protein 70.

- ▶ Chaperones
- ▶ Protein Trafficking and Quality Control

HSV

Synonyms

Herpes Simplex Virus

Definition

Acute infection with Herpes simplex viruses (HSV) results in painful rashes on skin and mucous membranes. HSV-1 mainly causes cold sores around the mouth (herpes labialis) or eyes (keratitis), whereas infection by HSV-2 mostly results in sores in the genital or anal area. Less frequently, HSV also causes severe infections in newborns or potentially fatal encephalitis. HSV remains latent and can be reactivated by stress, suppression of the immune system or other infections.

- ▶ Antiviral Drugs

5-HT

5-Hydroxytryptamine (serotonin, enteramine).

- ▶ Serotonergic System

Human Leucocyte Antigens (HLA)

The human leucocyte antigen (HLA) system is the general name of a group of genes in the human major histocompatibility complex (MHC) region on human chromosome 6 (mouse chromosome 17) that encodes the cell-surface antigen-presenting proteins.

- ▶ T Cell Receptors
- ▶ Immune Defense

Humanized Monoclonal Antibodies

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Definition

Monoclonal antibodies (mAb) are molecules that recognize and bind a specific foreign substance called an **▶ antigen**. They are produced from a single clone of **▶ B lymphocytes**. Conventionally, mouse mAb have been generated for experimental and diagnostic use. Techniques have been developed to humanize mouse mAb to facilitate their therapeutic use in humans. It is also now possible to make mAb which are fully human.

Mechanism of Action

Antibodies perform a key role in the vertebrate immune system recognizing specific foreign antigens and directing a targeted, adaptive, immune response designed to protect the host from a wide variety of pathogens. Antibodies are also known as immunoglobulins. They are large, Y-shaped **▶ glycoproteins** produced by specialized white blood cells known as B lymphocytes. Each B lymphocyte expresses immunoglobulin mAb on its cell surface where it acts as a receptor for one specific antigen. Following antigen recognition through surface immunoglobulin, B lymphocytes may differentiate and multiply into clones of antibody forming cells known as plasma cells. Plasma cells produce and secrete large quantities of antibody in soluble form. The antigen specificity is identical to the membrane-bound surface immunoglobulin receptor. Within the immune system, billions of antibodies with differing antigen specificities are produced. Individual B lymphocytes, or a clone of B lymphocytes, however, produce antibody of a single specificity only. Antibodies produced from clones of an

individual B lymphocyte will have identical antigen specificity and are known as mAb.

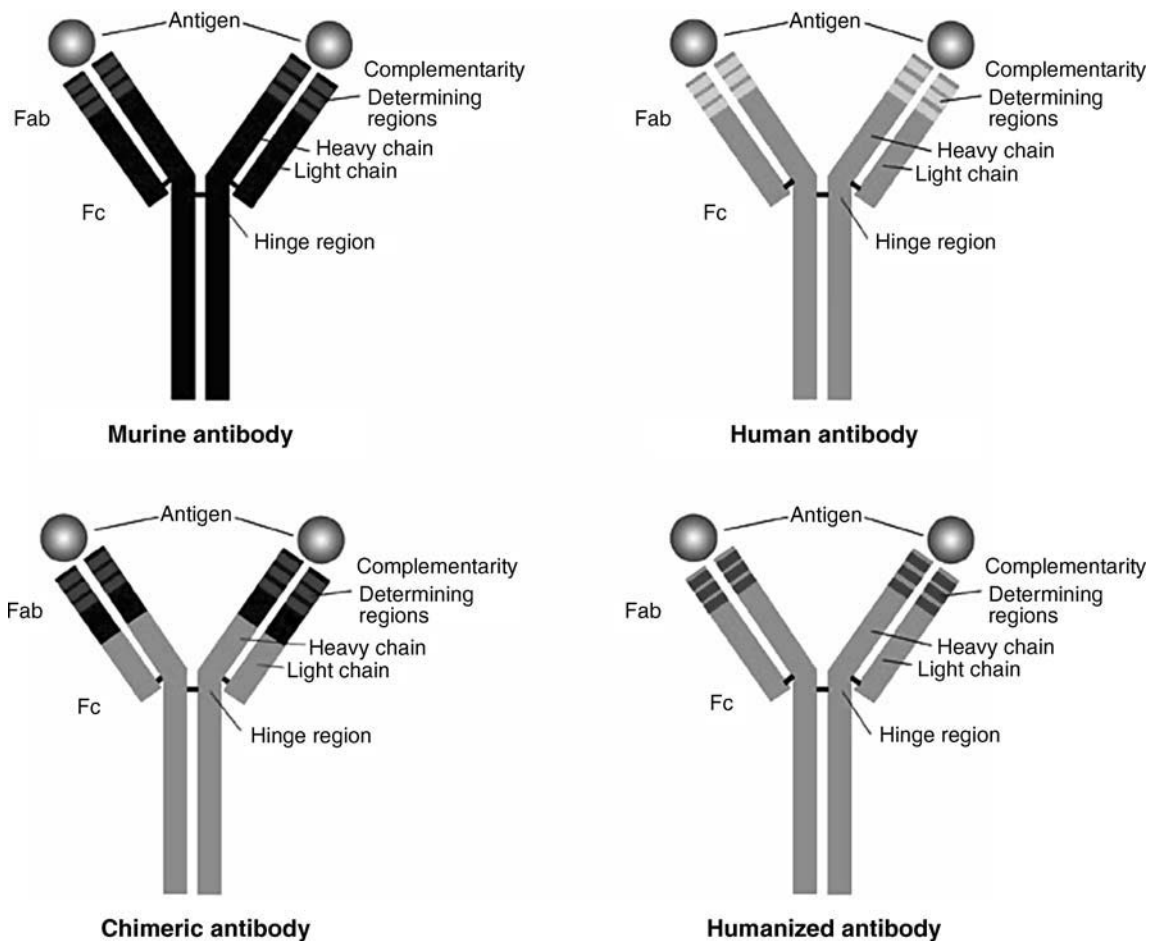
All antibodies possess the same basic Y-shaped structure comprised of two identical heavy and two identical light polypeptide chains linked together by a series of non-covalent and covalent disulphide bonds. Both light and heavy chains are folded into discrete regions called domains. Light chains exist in two different forms called κ and λ . Five distinct classes of antibody IgA, IgD, IgE, IgG and IgM are recognized in most vertebrates with antibody class being determined by heavy chain type, (α , δ , ϵ , γ or μ , respectively). Antibodies are bifunctional molecules: The arms at the top of the Y structure are primarily concerned with antigen binding and are known as Fab fragments. The amino acid sequences that make up part of each Fab fragment are characterized by sequence variability and are therefore referred to as variable regions. Within each variable region, three short polypeptide sequences show immense variability. These hypervariable regions together create the specific antigen binding site and as such are referred to as complementarity determining regions (CDRs). The intervening peptide fragments between the CDRs within the variable region act as a scaffold for the CDRs and are known as framework regions. The two Fab fragments are joined at an area of structural flexibility known as the hinge region. The remainder of the antibody molecule (the stem of the Y) is known as the Fc fragment and has a relatively constant structure. The Fc fragment is responsible for mediating immune effector functions such as complement activation or [phagocyte](#) binding following antigen recognition.

Antibodies are highly specific, binding only to a restricted part of a given antigen known as an epitope. Given the billions of antibody specificities that may be produced by the immune system, an antibody that recognizes an epitope on virtually any molecule may be produced. It is this property that makes antibodies immensely powerful tools for experimental, diagnostic and therapeutic procedures.

In the laboratory, an antibody may be raised by injecting antigen into an animal and then collecting the resultant antibody rich serum. However, this antiserum will contain a variable heterogeneous polyclonal mixture of antibodies produced by any number of different B lymphocytes that recognize a variety of epitopes present on the given antigen. In contrast, antibodies produced from identical copies or clones of a single B lymphocyte will have identical antigen epitope specificity and are known as mAb. In 1975, Köhler and Milstein won the Nobel Prize for devising an experimental technique enabling stable and permanent production of mAb. Mice were injected with an antigen of interest thereby eliciting an antibody response. Antibody producing B lymphocytes (isolated

from these animal's spleens) were then fused with cells previously derived from an immortal B lymphocyte tumour (myeloma). Hybrid cells (hybridomas) with the ability to make the specific antibody of interest and multiply indefinitely were then selected out, propagated and cloned, thereby providing a permanent and pure source of mAb from a single progenitor cell. Because mouse B lymphocytes are used to derive these hybridomas, the mAb produced by this system is of mouse origin (murine). Although murine mAb have proven to be of immense importance in experimental and diagnostic techniques, their use in human therapy is problematic. Firstly, murine antibodies may be recognized by a patient's immune system as foreign, leading to the generation of a human anti-mouse antibody (HAMA) response. HAMA responses may adversely affect the clinical efficacy and half-life of the antibody but also cause the clinical symptoms of [serum sickness](#). Secondly, because the Fc fragment of the mAb is murine rather than human, it may fail to activate appropriate immune effector functions, and as such, prove ineffective ([Figure 1](#)).

It is clearly unacceptable to immunize humans to raise antibodies and technically it has proven difficult to produce fully human antibodies by cell fusion techniques. Therefore, in an attempt to overcome the HAMA response and improve the clinical efficacy of murine derived mAb for therapy in humans, genetic engineering techniques have been developed to "humanize" murine antibodies. Chimeric antibodies with mouse variable regions and human constant regions may now be produced. This technique involves cloning the genes that encode for mouse antibody variable regions (specific to an antigen of interest) and inserting them into a vector along with the appropriate genes encoding for human immunoglobulin constant domains. This vector is then transfected into an appropriate cell line such as Chinese hamster ovary cells. These cells are then screened and cloned like conventional hybridomas. Colonies that secrete the resultant chimeric antibody can then be propagated, thereby providing a stable source of chimeric mAb. This humanization concept can be taken a step further to produce "CDR grafted" mAb. This approach requires the synthesis of a totally novel variable region using gene sequence information for the three epitope specific CDRs of interest from the variable regions of a mouse mAb, with compatible sequences from human variable framework regions. These humanized variable regions can then be linked to human constant region genes that, when expressed in an appropriate cell line, may produce a humanized mAb. In practice, the grafting of murine CDRs alone may result in some loss of antigen binding affinity, and a number of framework region amino acid residues may also need to be reintroduced alongside the CDRs to maintain antigen affinity. The result of both



Humanized Monoclonal Antibodies. Figure 1 Schematic representation of mouse, human, chimeric and

these approaches is to produce a mAb for human therapy that is specific to the antigen of interest, is less immunogenic, has a longer biological half-life and is more efficient at recruiting human Fc dependent immune effector functions.

An alternative technique for producing fully human mAb is the use of phage display libraries. Gene segments encoding for human variable domains of antibodies may be fused to genes that encode for ▶bacteriophage coat proteins. Bacteria may then be infected with the bacteriophage and the resultant phage particles that are produced will have coats that express the appropriate variable domain proteins. In this manner, phage display libraries may be built up consisting of a large collection of phages ($>10^{10}$), each expressing a different variable domain specific for a different antigen. Phage display libraries may then be challenged with an antigen of interest and phages that express appropriate antigen binding domains isolated and cloned. The gene encoding the variable region of interest may then be recovered from the isolated phage,

joined to the remaining parts of a human immunoglobulin gene and transfected into an appropriate host cell capable of antibody secretion. The result is production of a fully monoclonal human antibody with appropriate antigen specificity. One final approach to produce fully human mAb is to utilize transgenic animals. This technology requires deletion of an animal's own immunoglobulin genes and the subsequent introduction of human immunoglobulin gene segments. Subsequent antigen immunization then results in the generation of a fully human immunoglobulin.

mAb are thought to exert their therapeutic effect through a variety of mechanisms: In the treatment of conditions such as cancer, mAb may selectively bind to antigen on cells of interest, thereby targeting them for destruction through recruitment of the host's own immune system. Secondly, mAb may act by blocking cellular communication, a mechanism thought to be important in the treatment of some autoimmune inflammatory conditions. At the molecular level, this effect might be achieved by binding and disabling

cell surface receptors, or by binding and inactivating the signalling molecules themselves. Finally, mAb may provide their own inhibitory or stimulatory signals on binding cell surface receptors, a mechanism also thought to be of importance in mAb cancer therapy.

Clinical Use (Including Side Effects)

A number of chimerized, humanized, and one human mAb have now been approved for therapeutic use in humans in the treatment of autoimmunity, malignancy, infection and cardiovascular disease (Table 1). Some of the currently licensed mAb will be discussed here. A much larger number of mAb are currently being evaluated in Phase I, II and III trials. In general, chimeric, humanized and human mAb are very well tolerated with few side effects. Chimeric or humanized mAb still have the potential to evoke host immune response to the variable domains or CDRs of the antibody; so-called HACA (human anti-chimeric antibody) or HAHA (human anti-human antibody) responses, although these responses are uncommon. Short-lived and occasionally severe infusion-related acute hypersensitivity reactions such as fever, skin itching, shivering, respiratory compromise and low blood pressure sometimes occur. Such effects may

be abrogated by slowing the infusion rate or by co-administration of antihistamines and steroids.

Infliximab, a chimeric mAb against tumour necrosis factor (TNF) α has proven beneficial in the treatment of inflammatory diseases such as Crohn's disease and rheumatoid arthritis. TNF is a ►cytokine with a broad spectrum of pro-inflammatory biological activities. Infliximab binds TNF thereby blocking its activity. Clinical trials have clearly demonstrated significant clinical efficacy compared to placebo or conventional therapy. TNF blockade by Infliximab has been associated with a number of specific side-effects, notably an increase in opportunistic infections and the development of other autoimmune syndromes. Delayed hypersensitivity manifested by joint and muscle aches, rash and facial swelling have been reported rarely in patients retreated with Infliximab after a considerable treatment-free interval. Adalimumab is a more recently developed fully human anti-TNF. As of July 2006, it had been approved for the treatment of rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis. Basiliximab, a chimeric mAb, and Daclizumab, a humanized IgG mAb, are two mAb that are thought to exert at least some of their clinical effects through cell signal blockade. They are both licensed for the

Humanized Monoclonal Antibodies. Table 1 Major monoclonal antibodies FDA approved for clinical use

Monoclonal antibody	Mechanism of Action	Disease
Rituximab	Anti-CD20	B-cell NHL
Tositumomab	Radiolabelled anti-CD20	Follicular NHL
Ibritumomab	Radiolabelled anti-CD20	B-cell NHL
Gemtuzumab	Anti-CD33	AML
Alemtuzumab	Anti-CD52	CLL and T-cell lymphoma
Trastuzumab	Anti-HER2	Breast cancer
Cetuximab	Anti-EGFR	Colorectal cancer, Head and Neck cancers
Bevacizumab	Anti-VEGF	Colorectal cancer
Abciximab	Glycoprotein IIb/IIIa antibody	Coronary angioplasty
Infliximab	Anti-TNF α	Inflammatory bowel disease, rheumatoid arthritis, ankylosing spondylitis
Adalimumab	Anti-TNF α	Rheumatoid arthritis, ankylosing spondylitis
Eculizumab	Anti-complement protein C5	Paroxysmal nocturnal haematuria
Omalizumab	Anti-IgE	Asthma
Efalizumab	Anti-CD11a	Psoriasis
Ranibizumab	Anti-FAB fragment of VEGF	Macular degeneration
Basiliximab	Anti-IL-2 CD25	Allogeneic organ transplantation
Daclizumab	Anti-IL-2 CD25	Allogeneic organ transplantation
Palivizumab	Anti-RSV glycoprotein	Viral infections

suppression of graft rejection following allogeneic organ transplantation. Both mAb are specific for the interleukin-2 (IL-2) receptor (CD25) expressed on activated T lymphocytes, blocking IL-2 receptor binding and its resultant T lymphocyte proliferative effect. Rituximab, another chimeric IgG mAb, binds to CD20, a transmembrane protein present on normal and malignant B lymphocytes. It has proven efficacy in non-Hodgkin's lymphoma and has been shown to be effective both as a single agent and in combination with standard chemotherapy. Early clinical trials are also beginning to show impressive therapeutic activity in some autoimmune conditions, such as rheumatoid arthritis. Rituximab probably works through a variety of mechanisms that include immune effector targeting and receptor signalling. Ibritumomab tiuxetan – the murine parent anti-CD20 antibody of rituximab – is a mAb in conjunction with a chelator (tiuxetan) to which radioactive isotope is added (either yttrium-90 or indium-111). Treatment of B-cell NHL with Ibritumomab showed higher response rates in clinical trials compared to treatment with rituximab alone and in addition, demonstrated efficacy in patients who were no longer responding to rituximab. Trastuzumab, a humanized IgG mAb directed against the extracellular domain of the HER2 (ErbB-2) epidermal growth factor, has proven efficacy in patients with breast cancer whose tumours overexpress the HER2 receptor. Its effect is likely to be mediated through a number of mechanisms that include signal blockade and immune effector recruitment. Again, the antibody is effective as a single agent and has been shown to provide significant additional benefit to cytotoxic chemotherapy. Of note, Trastuzumab, particularly in combination with certain chemotherapeutic agents, has been associated with longer term cardiac dysfunction. The basis for the observed cardiotoxicity is, as yet, not fully explained.

Cetuximab is a chimeric mAb that targets the epidermal growth factor receptor (EGFR) which may be overexpressed on the surface of cancer cells. It is licensed for the treatment of metastatic colorectal, and head and neck cancers. Common side effects include acne-like rash, dry skin, lethargy, fever, constipation and abdominal pain. A second drug licensed for use in metastatic colorectal cancer is Bevacizumab, a humanized mAb that targets vascular endothelial growth factor (VEGF). VEGF is a protein that stimulates new blood vessel formation and it has recently emerged as a key player in tumour angiogenesis. VEGF expression is induced by hypoxia and glucose deprivation and it is almost universally expressed in tumours. Phase III randomized trials have demonstrated that the addition of bevacizumab to standard chemotherapy can improve overall survival in colorectal and lung cancer patients and progression free survival in patients with breast

cancer. The most common side effect is hypertension, but more serious adverse effects include thromboembolic events and gastrointestinal perforation. A modified fragment of the bevacizumab antibody has been developed and recently approved for use in macular degeneration (ranibizumab). Reports from clinical trials have shown substantially better outcomes in patients with choroidal neovascularization than conventional treatments such as laser or photodynamic therapy. Abciximab is the Fab fragment of a chimeric mAb against the platelet glycoprotein IIb/IIIa receptor. This receptor is involved in the final common pathway of platelet aggregation and thrombus formation, and Abciximab can successfully inhibit receptor function. Abciximab also binds to the vitronectin receptor found on platelets and endothelial cells inhibiting its pro-coagulant function. As an adjunct to aspirin and heparin, it has proven beneficial in the treatment of high risk patients undergoing percutaneous transluminal revascularization procedures for coronary artery disease, reducing the need for subsequent revascularization and the risk of myocardial infarction and death. Bleeding and a low platelet count are recognized complications of therapy. Finally, mAb have been developed to treat infective diseases. One example, Palivizumab, is a humanized mAb directed against an epitope on the fusion glycoprotein of the respiratory syncytial virus (RSV). It has potent neutralizing and fusion inhibitory activity against RSV. It is licensed for the prophylactic treatment of infants at high risk of infection with this virus and randomized trials have proven clinical benefit compared to placebo.

mAb have revolutionized experimental and diagnostic laboratory techniques. The humanization of murine mAb and the ability to make fully human reagents has allowed the development of a wide variety of generally safe, clinically useful, therapies. It seems likely that the humanization approach may well be superseded by the production of fully human antibodies produced by phage display techniques or through the use of transgenic animals. Given the large number of mAb currently undergoing pre-clinical development and being evaluated in clinical trials, the indications for mAb therapy are set to increase dramatically.

► Immune Defense

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Humoral Immunity

Humoral immunity depends on soluble, noncellular effector mechanisms of the immune system. These include ►defensins and ►complement components (proteins of the innate immune system) and ►antibodies (products of the adaptive immune system). They are capable of reacting with foreign substances (e.g., bacteria and viruses) to produce detoxification and elimination.

►Immune Defense

HVA Ca^{2+} Channels

Voltage-dependent Ca^{2+} channels that are activated at a membrane potential around -30mV with a maximal inward current around 0mV .

►Voltage-dependent Ca^{2+} Channels

Hybridization

The act of treating the array with one or more labeled preparations under a specified set of conditions, in order to bind complementary pairs of DNA molecules.

6-Hydroxydopamine

Dopaminergic neurotoxin that causes parkinsonism via lesion of nigrostriatal dopamine neurons in rat, mice, monkeys. Unlike the dopaminergic neurotoxin ►MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) it does not cross the blood–brain barrier.

►Monoamine Oxidases and their Inhibitors

Hydroxypyridones

►Antifungal Drugs

11 β -Hydroxysteroid Dehydrogenase Type II

11 β -hydroxysteroid dehydrogenase type II (11 β -HSD II) is a steroid metabolizing enzyme which is specifically expressed in epithelial tissues such as kidney or colon. 11 β -HSD II is an NAD^+ -dependent enzyme which has a low K_m for physiological glucocorticoids. The reaction in the dehydrogenase direction is essentially irreversible. 11 β -HSD II converts active glucocorticoids into their inactive ketoform, e.g. cortisol into cortisone. Since aldosterone possesses a cyclic 11,18-hemiacetyl-group, it is not a substrate for 11 β -HSD II. Thus, this mechanism ensures protection of mineralocorticoid target tissues from transcriptional activation by glucocorticoids. Mutations in the 11 β -HSD II gene are responsible for the syndrome of apparent mineralocorticoid excess.

►Glucocorticoids
►Epithelial Na^+ Channel
►Gluco-mineralocorticoid Receptors

5-Hydroxytryptamine (5HT)

Serotonin

►Serotonergic System

Hyoscine (Scopolamine)

Antagonist at muscarinic receptors.

►Muscarinic Receptors

Hyperactivity Disorder

► Psychostimulants

Hyperaldosteronism

Hyperaldosteronism is a syndrome caused by excessive secretion of aldosterone. It is characterized by renal loss of potassium. Sodium reabsorption in the kidney is increased and accompanied by an increase in extracellular fluid. Clinically, an increased blood pressure (hypertension) is observed. Primary hyperaldosteronism is caused by aldosterone-producing, benign adrenal tumors (Conn's syndrome). Secondary hyperaldosteronism is caused by activation of the renin-angiotensin-aldosterone system. Various drugs, in particular diuretics, cause or exaggerate secondary hyperaldosteronism.

- Aldosterone
- Diuretics

Hyperalgesia

Increased responsiveness to noxious stimuli is termed hyperalgesia. It occurs following injury or disease and encompasses enhanced responses as well as reduced thresholds to a given noxious stimulus. 'Primary' hyperalgesia occurs in the damaged area whereas 'secondary' hyperalgesia occurs in the area surrounding it.

► Pain and Nociception

Hypercholesterolemia

Elevated levels of cholesterol in the circulation.

- HMG-CoA-Reductase Inhibitors
- Low-Density Lipoprotein Receptor Gene Family

Hyperekplexia

Hyperekplexia (startle disease, stiff baby syndrome; OMIM databank: #138491) is a congenital human motor disorder that follows autosomal-recessive and dominant traits. Affected patients suffer from exaggerated startle responses to unexpected acoustic and tactile stimuli as well as episodic muscle stiffness. The startle response may trigger a sudden loss of postural control resulting in immediate and unprotected falling. In addition to profound startle responses, affected neonates exhibit a severe muscular hypertonia which may cause fatal apneic attacks. Muscle tone normalizes during infancy, while excessive startling persists throughout life. The clinical appearance varies considerably even within families with more than one afflicted member. A variety of mutant alleles of the glycine receptor subunit genes GLRA1 and GLRB have been found to cause hyperekplexia. These mutations affect glycine receptor affinity and ion conductance, or represent null alleles.

► Glycine Receptors

Hyperglycaemia

Elevated level of glucose in the blood.

► Diabetes Mellitus

Hyperinsulinemic Hypoglycemia of Infancy (HI)

HI usually presents at birth or within the first year of life and is characterized by excessive insulin release independent from blood glucose. Most cases of HI are sporadic with an estimated incidence of 1 in 27,000 live births in Ireland and 1 in 50,000 in Finland. However, in some isolated communities the disease incidence is much higher, e.g. 1 in 3,200 in the central area of Finland and 1 in 2,500 in the Arabian peninsula. Mutations in 5 different genes account for HI: the K_{ATP} channel subunits SUR1 (ABCC8) and Kir6.2 (KCNJ11) and the metabolic enzymes glucokinase

(GCK), glutamate dehydrogenase (GLUD1), and short-chain 1-3-hydroxyacyl-CoA dehydrogenase (SCHAD). Mutations in SUR1 (ABCC8) are the most common cause of HI (for details see ►ATP-dependent K^+ channel). Gain-of-function mutations in the genes for the glycolytic enzyme glucokinase (GCK) and the mitochondrial enzyme glutamate dehydrogenase (GLUD1) produce HI by increasing ATP synthesis. As a result, K_{ATP} channel activity decreases and insulin secretion is stimulated. Usually, mutations in GCK and GLUD1 produce a mild form of HI that does not require pancreatectomy.

►ATP-dependent K^+ Channel

Hyperkalemia

Hyperkalemia is an excess of potassium in the blood. Clinical symptoms are muscle weakness and cardiac arrhythmias. It is caused by, e.g., hyperaldosteronism and angiotensin-converting enzyme (ACE) inhibitors.

►Renin-Angiotensin-Aldosterone System
 ►Epithelial Na^+ Channels
 ►ACE Inhibitors

Hyperlipidaemia/ Hyperlipoproteinaemia

►HMG-CoA-Reductase-Inhibitors
 ►Lipoprotein Metabolism
 ►Lipid Transfer Proteins

Hyperplasia

Hyperplasia is the enlargement of an organ due to an increased number of cells.

►Growth

Hyperpolarization-activated and Cyclic Nucleotide-gated Channels (HCN)

►Cyclic Nucleotide-regulated Cation Channels

Hyperpyrexia

►Fever

Hypersensitivity

Hypersensitivity (or allergy) describes an inappropriate immune response to foreign substances, allergens, giving rise to irritant or harmful reactions.

►Allergy

Hypertension

Hypertension is a chronic elevation of blood pressure which is a major modifiable risk factor for cardiovascular and renal disease. There is no specific level of blood pressure where clinical complications start to occur; thus the definition of hypertension is arbitrary but needed in clinical practice for patient assessment and treatment. The diagnosis of hypertension in adults is made when the average of two or more diastolic blood pressure measurements on at least two separate visits is ≥ 90 mmHg or when the average of multiple systolic blood pressure readings on two or more separate visits is consistently ≥ 140 mmHg. Isolated systolic hypertension is defined as systolic blood pressure ≥ 140 mmHg and diastolic blood pressure < 90 mmHg. Essential, primary, or idiopathic hypertension is defined as high blood pressure in which causes of secondary hypertension or monogenic (mendelian) forms are not present. Essential hypertension accounts for 95% of all cases of hypertension. This condition is a

heterogeneous disorder, with different patients having different causal factors that lead to high blood pressure and usually requires pharmacological treatment.

- ▶ Blood Pressure Control
- ▶ Antihypertensive Drugs

Hyperthyroidism

Hyperthyroidism (thyrotoxicosis), defined as excessive thyroid activity, causes a state of thyroid hormone excess (thyrotoxicosis) characterized by an increased metabolic rate, increase in body temperature, sweating, tachycardia, tremor, nervousness, increased appetite and loss of weight. Common causes of hyperthyroidism are toxic multinodular goiter, toxic adenoma or diffuse toxic goitre (▶ Graves' disease). ▶ Antithyroid drugs (methimazol, carbimazole, propylthiouracil) block thyroid hormone production and are hence suitable for the treatment of hyperthyroidism.

- ▶ Antithyroid Drugs

Hyperuricemia

Hyperuricemia is defined as serum uric acid concentration $>416 \mu\text{mol/L}$ or 7.0 mg/dL . With increasing serum uric acid concentration, the risk of acute gouty arthritis increases, but asymptomatic hyperuricemia does not have to be treated pharmacologically.

- ▶ Anti-gout Drugs

Hypnotic

A medication that causes induction of sleep. The majority of currently available hypnotics (for example benzodiazepine receptor agonists) act via potentiating the brain's inhibitory GABAergic systems, in turn reducing the activity of arousal (i.e. wake promoting) neurotransmitter systems.

- ▶ Sleep
- ▶ GABAergic System
- ▶ Benzodiazepines

Hypoaldosteronism

Hypoaldosteronism is defined as a deficiency of aldosterone. Renal secretion of potassium is decreased, causing hyperkalaemia. The treatment is replacement of a mineralocorticoid, e.g. fludrocortisone.

- ▶ Aldosterone
- ▶ Gluco-mineralocorticoid Receptors

Hypocretins

Synonyms

Orexins

- ▶ Orexins

Hypoglycaemia

Hypoglycaemia is a reduction of blood glucose concentration to below normoglycaemia (euglycaemia). Severe hypoglycaemia will deprive the brain of adequate glucose (neuroglycopenia), which causes impaired neural function and can result in coma and death. Sulphonylureas, prandial insulin releasers and insulin can cause hypoglycaemia if administered in excess or if taken without appropriate food consumption.

- ▶ Oral Antidiabetic Drugs
- ▶ Insulin Receptor
- ▶ ATP-dependent K^+ Channels

Hypogonadotropic Hypogonadism

Hypogonadotropic hypogonadism is a genetically heterogeneous disorder characterized by infertility and

absent or incomplete pubertal maturation. If the gene for the ►GnRH receptor is affected by loss of function mutations, a complete GnRH-resistant form of the disease is observed.

- Protein Trafficking and Quality Control
- Gonadotropin-releasing Factor/Hormone (GnRH)

Hypokalemia

Hypokalemia is a reduction of plasma K^+ concentration below 3.5 mM. Hypokalemia can result from a reduction in dietary K^+ intake and from a shift of K into the intracellular space. The most common of hypokalemia, however, is renal K^+ loss (i.e., caused by diuretics).

- Diuretics
- Hyperaldosteronism
- Epithelial Na^+ Channels

Hypomethylating Agents

DNA hypomethylating agents like decitabine stop the methylation of DNA by eliminating the enzyme DNA methyltransferase 1, which adds methyl groups to CpG sites of DNA. Increased methylation of CpG sites in the promotor area of a gene is related to the gene's gradual shut down. Decitabine and other hypomethylating agents work by removing the methyl groups, enabling the gene to become active again, which is vital in the case of tumor-suppressor genes, which normally protect cells from becoming cancerous.

- Antimetabolites
- Epigenetics
- DNA Methylation

Hyposensitization

Hyposensitization is an empirically founded immunotherapy which is characterized by repeated exposure to the responsible (clearly defined) allergen. It can lead to a diminished Type I (IgE-dependent) allergic reaction.

- Allergy

Hypotension

Hypotension is defined as abnormally low blood pressure. In most cases, hypotension is adequately treated with general measures (e.g. physical exercise), drug treatment is rarely required. Drugs used for the treatment of hypotension include α -adrenoceptor agonists and compounds which activate both α and β adrenoceptors.

- α -Adrenergic System
- β -Adrenergic System
- Kinins

Hypothalamus

Brain structure below the thalamus and main portion of the ventral region of the diencephalon, controlling homeostatic and nonhomeostatic basic body and brain functions, including circadian and feeding rhythms, energy metabolism, thermogenesis, sympathoadrenal, and neuroendocrine outflow (secretion of hormones by the pituitary gland), behavioral state and memory functions.

- Hypothalamus-pituitary-adrenal (HPA) Axis
- Circadian Rhythms
- Appetite Control
- Orexins
- Antiobesity Drugs

Hypothalamus–Pituitary–Adrenal (HPA) Axis

Regulatory circuit controlling glucocorticoid secretion and synthesis by the adrenal gland. In an endocrine cascade, corticotropin releasing hormone (CRH) in the hypothalamus stimulates adrenocorticotrope hormone (ACTH) in the anterior pituitary, which then acts on the adrenal gland in order to release glucocorticoids. In a negative feedback loop, the hormone inhibits the HPA axis via binding to glucocorticoid receptor and mineralocorticoid receptor, thus ensuring homeostasis of glucocorticoid serum levels.

- ▶ Gluco-mineralocorticoid Receptors
- ▶ Glucocorticoids
- ▶ Antidepressant Drugs

during development causes cretinism, characterized by retardation of growth and mental deficiency.

- ▶ Antithyroid Drugs

Hypothyroidism

Decreased activity of the thyroid gland results in hypothyroidism and, in severe cases, myxoedema. It is often of immunological origin and the manifestations are low metabolic rate, slow speech, lethargy, bradycardia, increased sensitivity to cold, and mental impairment. Myxoedema includes a characteristic thickening of the skin. Therapy of thyroid tumours is another cause of hypothyroidism. Thyroid deficiency

Hypoxia

Lack of oxygen in blood or tissues. Tissue hypoxia can be caused by injury, inflammation, or tumor growth, due to disruption of blood supply. Tissue hypoxia is normally associated with acidosis, as anaerobic metabolism leads to production of lactic acid.

- ▶ Proton-sensing GPCRs
- ▶ Hematopoietic Growth Factors

IC₅₀ Values

The IC₅₀ value is that concentration of a drug that reduces the activity (or binding) of another drug to an enzyme by 50%. Under certain conditions it can be used to express the affinity of the enzyme inhibitor.

- Drug Interactions
- Drug-receptor Interaction

Idiopathic Hypercalciuria

Hypercalciuria is clinically defined as a daily urinary calcium excretion greater than 100 μM/kg, and it is associated with nephrocalcinosis, urinary stone formation, proteinuria, and renal failure. The genetic background is probably heterogeneous. Two hypercalciuric syndromes, Dent's disease and X-linked recessive nephrolithiasis, have been linked to mutations in the gene encoding a voltage-gated chloride channel (ClC-5).

- Cl⁻ Channels and Cl⁻/H⁺ Exchangers
- Diuretics

Idiosyncratic Reactions

An idiosyncratic reaction is a harmful, sometimes fatal reaction, that occurs in a small minority of individuals. The reaction may occur with low doses of drugs. Genetic factors may be responsible, e.g. glucose-6-phosphate dehydrogenase deficiency, although the cause is often poorly understood.

- Pharmacogenetics

IGF

- Insulin-like Growth Factor
- Insulin Receptor

IKK Complex (IKK Family)

The IκB kinase (IKK) complex is a high molecular weight (600–900 Kd) multisubunit complex present in the cytosol of most cell types. It contains two highly homologous catalytic subunits, IKKα (or IKK1) and IKKβ (or IKK2) that are serine/threonine protein kinases. They are structurally related and contain an amino-terminal kinase domain, a central leucine zipper region required for their dimerization and a carboxyl-terminal helix–loop–helix domain, which mediates protein interaction. The catalytic subunits interact through their carboxyl-terminal end with the third subunit, IKKγ (or NEMO for NF-κB essential modulator). IKKγ is the regulatory subunit of the IKK complex. It is structurally composed of protein–protein interaction motifs including two coiled–coiled domains, a leucine-zipper domain and a zinc-finger domain. IKKγ is essential for the structural organization and stability of the complex. IKKγ also has a regulatory function as it connects the IKK complex to potential upstream activators. The IKK complex phosphorylates IκBα, an inhibitor of NFκB, removing it from the NFκB dimer and allowing NFκB activation. The IKK complex is involved in Toll-like receptor signalling.

- Nuclear Factor Kappa B
- Toll-like Receptors

Ikr

Ikr is a rapidly activating component of the delayed rectifier potassium current. Ikr stands for potassium (K) rectifier (r) current (I). Ikr is characterized by delayed

activation and slow inactivation and contributes to repolarization phase of the action potential. Terikalant, originally thought as a highly specific blocker of I_{K1} channels, possesses even higher blocking affinity for I_{kr} than for I_{K1} .

- Inward Rectifier K^+ Channels
- K^+ Channels

Imidazoline Receptor

The imidazoline receptor is a hypothetical receptor for a subgroup of α_2 adrenergic agonists, which are characterized by their imidazoline structure (e.g. moxonidine). So far, there is no proof of the existence of imidazoline receptors.

- α -Adrenergic System

Immediate Early Genes

Immediate early genes, e.g., c-fos, c-jun, and c-myc, are the first genes whose expression is induced in cells after a growth stimulus. They encode transcription factors and induce the expression of other growth-related genes.

Immune Complexes

Immune complexes are aggregates of antibodies with their (foreign) antigens which physiologically initiate the clearance of the substance via ingestion and subsequent intracellular degradation by phagocytic leukocytes. In type III allergic reactions immune complexes cause disease.

- Allergy

Immune Defense

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Synonyms

Immunity

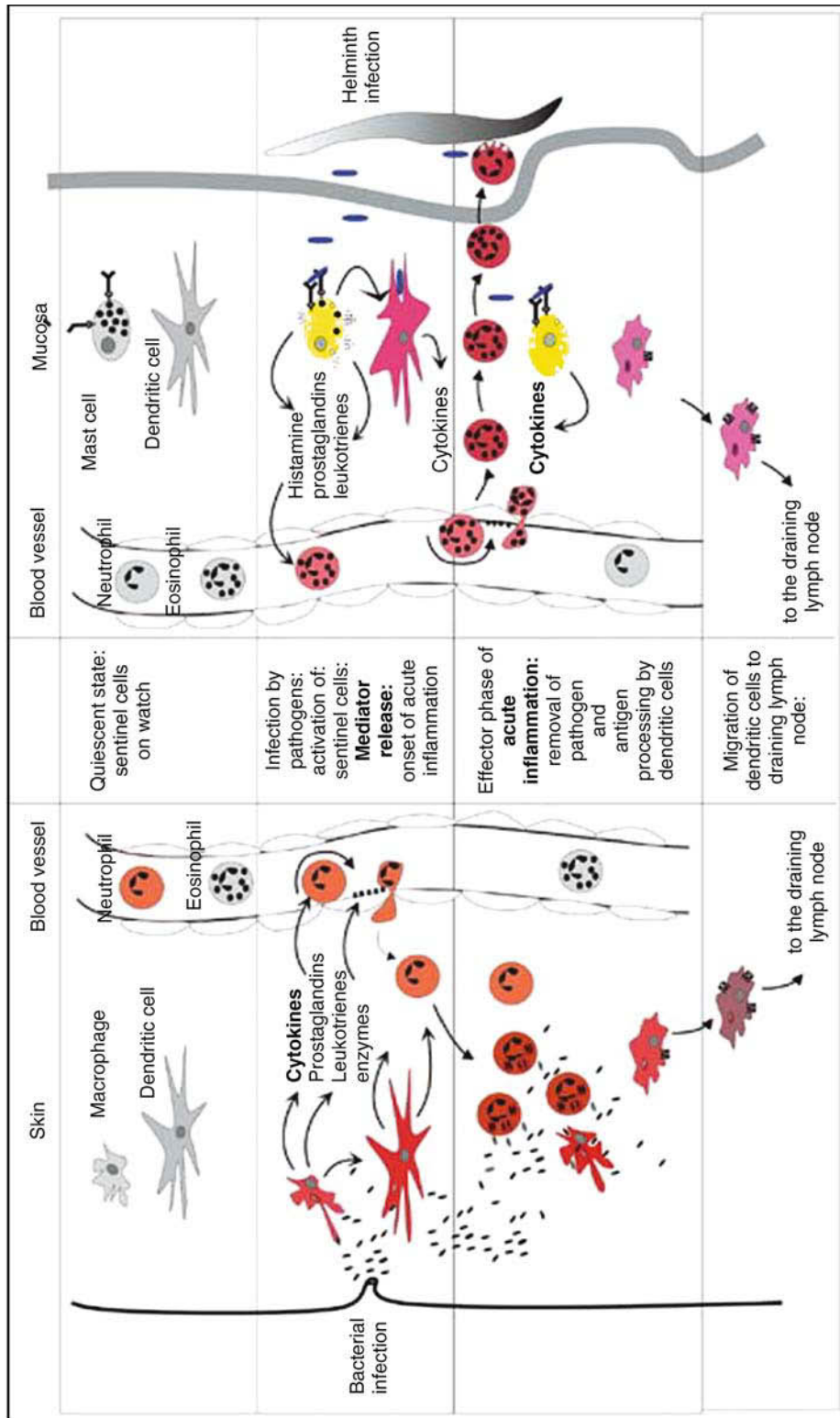
Definition

Immune defense is the ability of higher organisms to identify and combat potentially harmful microorganisms such as viruses, bacteria, fungi, protozoa, and helminths by highly sophisticated mechanisms involving soluble factors (► [Humoral Immunity](#)) and immune competent cells (► [Cellular Immunity](#)). In humans two arms of the ► [Immune System](#) exist that comprise the innate immune response and the adaptive immune response. The latter is characterized by a high specificity as an immunological memory-enabling adaptation to a given microbial environment. Mechanisms of immune defense are also utilized to identify and eliminate injured tissue or (neoplastic) tumor cells.

Basic Characteristics

Acute Inflammation is the Immediate Response of Innate Immunity to Pathogens.

The task of ► [innate immunity](#) is to respond rapidly to challenges by pathogens by mounting an acute inflammatory response (► [inflammation](#)) preferably at the site of infection in order to avoid a systemic spreading of pathogens (summarized in [Fig. 1](#)). Humoral components of innate immunity include the complement system, defensins, or antibacterial enzymes such as lysozyme. Cellular components of innate immunity comprise dendritic cells, monocytes/macrophages, mast cells, neutrophilic and eosinophilic granulocytes, basophils, and natural killer cells. Tissue macrophages, dendritic cells, and mast cells serve as sentinel cells at critical sites of pathogen entry such as skin and mucosa. They recognize structures from pathogens via pattern recognition receptors. Depending on the nature of the pathogen and its site of entry these sentinel cells release typical mediators including ► [cytokines](#), ► [chemokines](#), prostaglandins, leukotrienes, and in the case of mast cells, also histamine. Thus, dendritic cells and macrophages preferably recruit and activate neutrophilic granulocytes and mount an antibacterial response (left hand side of [Fig. 1](#)), Bacteria are ingested by phagocytosis and subsequently inactivated and degraded. Mast cells provide an environment enabling



Immune Defense. Figure 1 Innate immune responses after challenge of skin or mucosa: Acute inflammation and priming of adaptive immunity.

eosinophils to become activated to secrete effector molecules with the aim to combat pathogens too large to become phagocytosed such as helminths (right hand side of Fig. 1). The activated infiltrating leukocytes support the resident phagocytic cells by effectively removing and/or killing the pathogens (neutrophilic granulocytes and monocytes/macrophages) or by killing (virus-) infected host cells (NK cells).

While cells of the ►innate immune system can combat invading microbes autonomously, their effector mechanisms are also used and greatly enforced by the ►adaptive immune system. This includes enhanced complement activation and phagocytosis by antibodies as well as boosting the activation of phagocytic cells by cytokines such as interferon (IFN)- γ . On the other hand, innate immunity is indispensable for initiating an adaptive immune response.

The Dendritic Cell Is the Innate Link to Adaptive Immunity

Parallel to orchestrating acute inflammatory processes by providing an optimal milieu of cytokines, mediators, and adhesion molecules in order to recruit and activate effector cells to the site of infection, dendritic cells also serve as professional antigen-presenting cells for cells of the adaptive immune system (►antigen presentation; ►antigen receptors). After having ingested pathogens or pathogenic structures they move out of the (inflamed) tissue into the draining lymph node (bottom part of Fig. 1). During this migration they process antigens and present peptides from ingested particles, mostly from microbes on MHC class II molecules, which become upregulated.

Activation of the Adaptive Immune Response

In the specialized environment of secondary lymphoid tissues such as lymph nodes or spleen, dendritic cells provide the requirements for naive T-lymphocytes to become activated and to proliferate. The professional antigen-presenting cells present peptides in MHC II, express ►costimulatory molecules, and release cytokines into the immunological synapse, which is formed by the antigen-presenting cell and the naive T-lymphocyte. Thus, cells of innate immunity initiate and facilitate the activation of naive lymphocytes, and it is easily conceivable that their cytokines and adhesion molecules will instruct the naive T-lymphocyte during activation and differentiation to T-effector cells.

Cellular Components of Adaptive Immune Responses are T- and B-Lymphocytes whereas Humoral Components are Antibodies.

The hallmark of T- and B-lymphocytes is that each single lymphocyte expresses antigen receptors of a single specificity that was created randomly during the development of that individual lymphocyte. This is achieved mainly by sequential genetic rearrangement

of the gene segments coding for either the β - and α -chain of the T-cell antigen receptor or the heavy and light chain of the surface immunoglobulin, which serves as B-cell antigen receptor. In theory, this process is able to generate up to 10^{13} specificities, some of which can react with antigens of its own body. These self-reactive lymphocytes are eliminated by selection processes within the thymus (T-lymphocytes) or bone marrow (B-lymphocytes) establishing ►central tolerance. The remaining mature naive T- and B-lymphocytes, each carrying antigen receptors with single antigen specificity, create the immunological repertoire of about 10^8 antigen-specific lymphocytes in human beings. Upon encountering their specific antigen, lymphocytes proliferate – thereby expanding the antigen-specific cells (clone) – and become effector cells (►clonal selection). In strict contrast to T- and B-lymphocytes, cells of the innate immune system do not rearrange genes to create receptors recognizing pathogenic structures; innate immune receptors are always germline configured. In addition, cells of the innate immune system do not start to proliferate after activation.

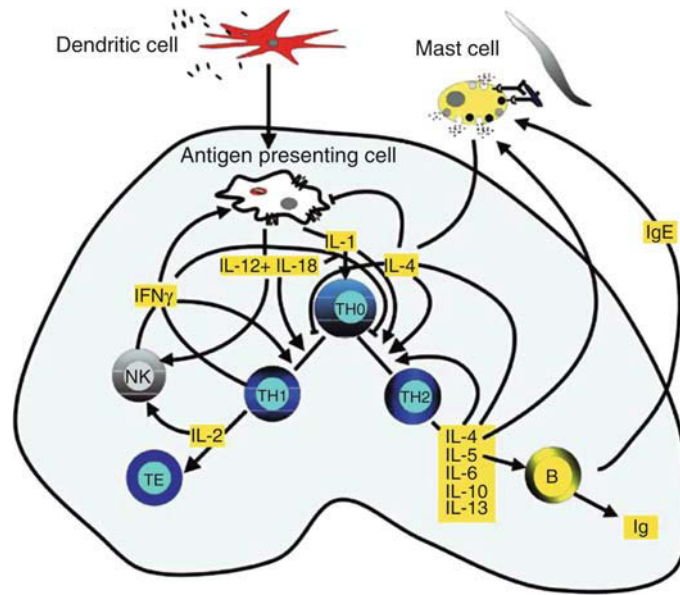
T-Lymphocytes

This class of lymphocytes differentiates from immunologically incompetent hematopoietic stem cells of the bone marrow within the thymus – hence, the name thymus-dependent (T-) lymphocytes. Two major subclasses develop simultaneously, T-helper lymphocytes (Th) and cytotoxic effector lymphocytes (Tc). The cytotoxic T-lymphocytes (carrying on the surface the differentiation marker CD8) destroy cells, which carry their cognate antigen bound to MHC class I molecules on the surface by inducing apoptosis. From an evolutionary point of view Tc cells appear to have developed predominantly to cope with virus infections. As viruses can only replicate within cells, Tc eliminate them by destroying their producers.

For an effective defense, antigen-specific Tc has to proliferate, whereby up to a 10^7 -fold increase in numbers may occur. This process is regulated by T-helper lymphocytes, which recognize their specific antigen when it is presented on MHC-class II molecules with their T-cell antigen receptor and CD4. A subpopulation, Th-1 cells, provides the central growth factor, interleukin-2 (IL-2). These cells also secrete IFN- γ , which represents the strongest activator of macrophages and thus recruits the innate immune system for adaptive immune responses, especially for the removal of bacteria or fungi.

A second population of Th-cells, Th-2, regulates B-cell responses (see below) by secreting a different set of cytokines including IL-4, IL-5, IL-6, IL-10, or IL-13.

Th-1 and Th-2 cells develop during an immune response from a common ancestor, the Th-0 cells. Th-1 cells, once generated, promote their own differentiation



Immune Defense. Figure 2 Cytokines involved in the development of adaptive immune responses in secondary lymphoid tissues such as the lymph nodes or spleen. Abbreviations: B: B-lymphocyte, IFN: interferon, Ig: immunoglobulin, IL: interleukin, NK: natural killer cell, TE: T-effector (cytotoxic) lymphocyte, TH: T-helper lymphocyte

and simultaneously block the development of Th-2 cells. Vice versa also Th-2 cells promote their own differentiation and inhibit that of their counterpart. In addition, Th-1 development is initiated by secretion of IL-12 and IL-18 from cells of the monocytic lineage, often stimulated by, e.g., bacteria, and Th-2 by secretion of IL-4 from mast cells. Thus the balance between Th-1 and Th-2 cells is central for a physiological defense mechanism, which usually affects T- as well as B-cell responses (schematically depicted in Fig. 2). If it is skewed toward Th-1, chronic inflammatory situations may occur, if it is skewed toward Th-2, allergy can be the result.

The Th-1/Th-2 paradigm forms a core mechanism regulating the nature of an immune response. More recently, this concept was further developed by identifying Th-subsets with predominantly suppressing properties, T-regulatory cells (Treg). These cells also play a major role in keeping those cells at rest, which have escaped central tolerance (peripheral self tolerance).

B-Lymphocytes

Also derived from the hematopoietic stem cells, B-lymphocytes differentiate in the bone marrow. Upon binding of an antigen to its immunoglobulin receptor a B-cell starts to proliferate and differentiate into plasma cells synthesizing and secreting antigen-specific antibodies. Initially high molecular weight IgM is secreted, upon continued or repeated antigen-binding the B-lymphocyte switches to other immunoglobulin classes, IgG, IgA, or IgE, which preserve the original antigen

specificity. These antibody classes fulfill different functions. IgM is very effective in activating complement that can kill bacteria and thus constitute an early defense mechanism against invading infectives. Because of its size, however, it does not penetrate into tissues. IgG, which is the most abundant immunoglobulin, can reach sites outside the circulation, it is less effective in complement activation, but additionally induces phagocytosis by interacting with immunoglobulin Fc-receptors on phagocytic cells. IgA is secreted into gut, mucosal membranes of the respiratory tract or tear ducts and constitutes an early barrier for invading infectives literally outside of the body. IgE has an important function in fighting infections that are too large to be ingested by cells, such as parasites. In highly industrialized countries, IgE is predominantly associated with allergy.

Effective antibody synthesis and switching from IgM to the other Ig-classes requires help by Th-cells, predominantly but not exclusive by Th-2 cells. The master cytokine responsible for a switch to IgE and thus development of an allergy is IL-4.

Drugs

Reasons to Intervene in Immune Defense Mechanisms

Immune defense mechanisms can become deleterious for an individual when they are not controlled properly. Then they can cause disease. In such situations therapy is aimed to dampen immune reactions. Important examples are septic shock, allergy, ► **autoimmune diseases**, and chronic inflammatory diseases such as rheumatoid arthritis. Also, the success of organ transplantation

depends on the inhibition of the immune response against the foreign organ.

However, several situations can be anticipated in which support of the immune system is required. These include congenital defects in the immune repertoire, acquired immune deficiencies such as in HIV infection, but also situations in which the immune system is compromised after treatment of patients, e.g., after radiation or chemotherapy.

Support of Immune Mechanisms

Vaccination

Probably the oldest and most efficient method of supporting immune defense mechanisms is vaccination. The principle is to induce an immune reaction toward an attenuated, dead, or denatured pathogen or parts of it in order to raise a high titer, high-affinity specific antibody response or to generate memory T-effector cells. Upon reexposure this will allow rapid and efficient clearance of the pathogen before it can cause harm. If such protective antibodies are generated by vaccination of the individual, one calls this active immunization. If the antibodies are raised in other individuals or animals and injected into the recipient for immediate protection this is denominated passive immunization. Vaccination has proven to be an extremely powerful and successful tool to combat many viral infections, with increasing success also in bacterial infections.

Tumor Cell Vaccination

Tumor cells are recognized and killed by cytotoxic T-cells. Unfortunately, frequently the cytotoxic response mounted against the tumor cells is not sufficient in patients to eliminate the tumor. The number and activation status of tumor-specific T-cells can be increased by tumor-cell vaccination. This technique uses dendritic cells derived from the patient that are loaded with tumor antigens by different methods. This results in the presentation of peptides of the tumor cells on the dendritic cell. These modified antigen-presenting cells are reintroduced into the tumor patient where they are capable of either mounting a nonexistent antitumor response by T-cells or by enhancing an existing but inefficient T-cell response to the tumor. This novel strategy is presently in an experimental stage with encouraging clinical results.

Use of Antibodies

Human immunoglobulin preparations from pools of a great number of people (>1,000) with assumed antibodies against common viruses are used as a means of "passive immunization" in acute infections. More specific antibody preparations with high titers from patients who recovered recently from a viral disease or were immunized against toxins are also available in

some countries. With the advent of monoclonal antibody technology a series of murine antibodies were probed with respect to therapeutic intervention. To avoid rapid formation of human antimouse antibodies, often non-antigen binding parts of the antibody molecule are replaced by human homologues (humanized monoclonal antibodies). The use of therapeutic antibodies is rapidly increasing, including antitumor antibodies, antibodies that prevent blood clotting, or inhibit immune reactions (see below).

Application of Recombinant Cytokines

Several cytokines are in clinical use that support immune responses, such as IL-2, IFNs, or colony-stimulating factors. IL-2 supports the proliferation and effector function of T-lymphocytes in immune compromised patients such as after prolonged dialysis or HIV infection. IFNs support antiviral responses or antitumoral activities of phagocytes, NK cells, and cytotoxic T-lymphocytes. Colony-stimulatory factors enforce the formation of mature blood cells from progenitor cells, e.g., after chemo- or radiotherapy (G-CSF to generate neutrophils, TPO to generate platelets, EPO to generate erythrocytes).

Unspecific Stimulation of the Immune Response

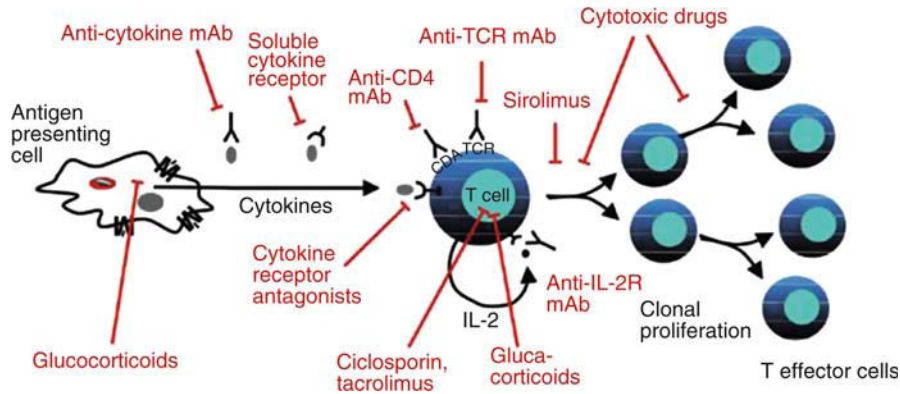
Dead or live bacteria may be effective to stimulate inflammatory reactions of phagocytic cells against tumor cells. The best-characterized treatment is the use of *Bacillus Calmette Guerin* (BCG) in the case of bladder cancer where activation of the immune response is capable of controlling tumor growth.

Similar results may be obtained with muramyl dipetides and CpG oligonucleotides or $Al(OH)_3$, which is commonly used as adjuvants in the course of vaccination.

Inhibition of Immune Mechanisms

Two main strategies are presently used to suppress immune responses (summarized in Fig. 3). The first focuses on cytokines, the central mediators of the immune system. Efficient inhibition of cytokine production can be achieved by glucocorticoids. Specific anticytokine strategies include the use of monoclonal antibodies, soluble receptors, or receptor constructs.

The second target is the T-lymphocyte as the central regulatory cell of specific or adaptive immunity. Inhibition of T-cell activation can be achieved on different levels. Drugs like ciclosporin or tacrolimus (FK506) affect activation, whereas cytostatic drugs or sirolimus (rapamycin) inhibit proliferation. Specific elimination of T-cells can be achieved by anti-CD3 antibodies or anti-CD4 antibodies, unspecific cell killing of proliferating T-cells by cytotoxic drugs. The



Immune Defense. Figure 3 Drugs involved in suppressing innate and adaptive immune response. Abbreviations: mob: monoclonal antibody, TCR: T-cell antigen receptor, IL-2 interleukin-2, R: receptor, CD: cluster of differentiation.

aim is to impair immune reactions by removing the source of T-cell help (►[immunosuppression](#)).

Glucocorticoids

At pharmacological concentrations glucocorticoids are the most efficient inhibitors of the synthesis of several cytokines, including those produced by T-lymphocytes, like IL-2, IL-4, or IFN γ , or by inflammatory cells, e.g., IL-1, IL-6, IL-8, IL-12, IL-18, or TNF α (►[cytokines](#)). The molecular mechanisms of the inhibition of cytokine protein synthesis – in contrast to their physiological action, i.e., the induction of metabolic enzymes (►[glucocorticoids](#)) – is not completely understood. Contributing factors are the interaction of the cytosolic glucocorticoid receptors after they have bound glucocorticoids with transcription factors regulating cytokine synthesis, and thereby blocking their binding to DNA, as well as the induction of inhibitory proteins. Glucocorticoids are effective in severe forms of allergy, autoimmune diseases, and all forms of chronic inflammatory diseases.

Anticytokine Regimens (Treatments)

In addition to inhibiting cytokine synthesis by glucocorticoids, cytokine effects can be prevented by scavenging the cytokine either with neutralizing antibodies or soluble receptors or by blocking the respective cytokine plasma membrane receptors with blocking antibodies or receptor antagonists.

Examples are:

- Anticytokine antibodies Infliximab: Chimeric (mouse/human) monoclonal antibody against TNF α . Effective in the treatment of severe forms of rheumatoid arthritis where it can halt disease progression, or inflammatory bowel disease (IBD).

- Adalimumab: Complete human antibody against TNF- α with similar properties as infliximab.
- Soluble receptor constructs Etanercept: This genetically engineered drug consists of the extracellular part of the TNF-receptor type I and the Fc portion of human IgG. Its application in rheumatoid arthritis mirrors that of infliximab.
- Anticytokine receptor antibodies Basiliximab, Daclizumab: Both are humanized monoclonal antibodies against the IL-2 receptor that block T-cell proliferation by inhibiting IL-2 and thus decrease the T-cell mediated frequency of rejection episodes in organ transplantation.
- IL-1 receptor antagonist Anakinra: A naturally occurring complete antagonist, effective in rheumatoid arthritis.

- [Allergy](#)
- [Autoimmune diseases](#)
- [Chemokine Receptors](#)
- [Cytokines](#)
- [Humanized Monoclonal Antibodies](#)
- [Immunosuppressive Agents](#)
- [Inflammation](#)
- [Interferons](#)
- [T Cell Receptors](#)

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Immune System

The immune system is a distinct organ in vertebrates, specialized to defend against invading infections or poisons in order to preserve the integrity of the organism. For this task it is disseminated throughout the body in primary and secondary lymphoid organs and the circulating blood. It contains the cells responsible for innate and adaptive immunity and humoral factors.

► Immune Defense

Immunity

Immunity is the innate or acquired ability of a higher organism to successfully defend life against potentially harmful agents like infections or poisons.

► Immune Defense

Immunoglobulins (Immunoglobulin Superfamily)

- Antibodies
- Humanised Monoclonal Antibodies
- Allergy
- Table appendix: Adhesion Molecules

Immunomodulators

Immunomodulators are a group of mostly stimulatory effectors which act on cells of the immune system (e.g. cytokines, interferons).

- Cytokines
- Interferons

Immunophillins

Immunophillins are abundant proteins that catalyze the *cis-trans* isomerization of proline residues within proteins, generally to aid in protein folding. Immunophillins are not essential proteins, are the intracellular binding proteins of several immunosuppressive drugs. Cyclosporin A exerts its action after binding to cyclophilin. Tacrolimus and sirolimus predominantly bind to the protein ►FKBP-12 (FK binding protein-12).

► Immunosuppressive Agents

Immunosuppressive Agents

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Synonyms

Immunosuppressants

Definition

Immunosuppressive agents (immunosuppressants) are drugs that attenuate immune reactions. An application is indicated in case our immune system reacts inadequately leading to serious diseases or normal immune reactions are unwanted, e.g., following transplantations.

Mechanism of Action

Immunosuppressive drugs comprise a large spectrum of substances with different mechanisms of action where ►T-lymphocytes represent a major target [1, 2]. In general, immunosuppressants can be divided into those that

- Inhibit T-lymphocyte proliferation in an unspecific manner
- Decrease the pool of circulating lymphocytes
- More specifically attenuate the activation of T-lymphocytes
- Inhibit the interaction between antigen or antigen-presenting cells (APCs) and T-lymphocytes

Antiproliferative Drugs Cyclophosphamide

This drug belongs to the group of alkylating antineoplastic drugs. Alkylation finally results in covalent crosslinkage of DNA strands that interrupts the

replication of all dividing cells including activated lymphocytes. Thus, both cellular and humoral immune reactions (antibody production) are inhibited.

Methotrexate Including its Polyglutamates

Methotrexate belongs to the class of antimetabolites. As a derivative of folic acid it inhibits the enzyme dihydrofolate reductase resulting in a decreased production of thymidine and purine bases essential for RNA and DNA synthesis. This interruption of the cellular metabolism and mitosis leads to cell death.

Inhibitors of De Novo Purine Biosynthesis

Inosine monophosphate dehydrogenase (IMPDH) is a key enzyme of purine nucleotide biosynthesis. Purine synthesis in lymphocytes exclusively depends on the de novo synthesis, whereas other cells can generate purines via the so-called “salvage pathway.” Therefore, IMPDH inhibitors preferentially suppress DNA synthesis in activated lymphocytes.

Azathioprine

In vivo azathioprine is rapidly converted into its active metabolite 6-mercaptopurine by the enzyme thiopurine methyltransferase (TPMT). The active agent inhibits IMPDH function. Furthermore, it also acts as antimetabolite of the RNA and DNA synthesis particularly in T-lymphocytes leading to cell death. Due to genetic polymorphism of TPMT, therapy may fail, thus it is currently discussed whether individual patients should be monitored before the use of azathioprine.

Mycophenolate Mofetil

The active metabolite of this drug is mycophenolic acid (MPA), which inhibits IMPDH, too. MPA is metabolized in vivo by glucuronidation. It has to be noted that its acyl glucuronide inhibits IMPDH with similar potency compared to the parent compound.

Mizoribine

This immunosuppressive drug, which is only marketed in Japan, is a nucleoside analog. Its phosphorylated form, mizoribine-5-phosphate, is a potent inhibitor of IMPDH activity.

Inhibitors of the De Novo Pyrimidine Biosynthesis

Leflunomide

The active metabolite of leflunomide, the ring-opened drug A771726, inhibits dihydroorotate dehydrogenase (DHOD) which is the key enzyme of the de novo pyrimidine synthesis. Inhibition of synthesis stops proliferation of activated lymphocytes. The leflunomide derivative FK778 which shows similar therapeutic efficacy but shorter half-life is investigated in clinical trials.

Brequinar

This drug also inhibits DHOD activity but has not been approved yet.

Immunosuppressive Drugs Acting by Lymphocyte Depletion

Antilymphocyte Globulin and Antithymocyte Globulin

Both globulins exert their effect by depletion of circulating lymphocytes either by complement-dependent lysis or by phagocytosis after opsonization. However, antilymphocyte globulin (ALG) and antithymocyte globulin (ATG) are nonhuman polyclonal antibodies. To prevent sensitization application is restricted to a time period of several days only.

Anti-CD3 (Muronomab-CD3)

Muronomab-CD3 is a murine monoclonal antibody directed against the CD3 complex of the T-lymphocyte antigen receptor. This drug selectively diminishes the T-lymphocyte pool resulting in a strong lymphopenia. Similar to other nonhuman antibodies the generation of human antimurine antibodies (HAMA) limits its long-term use.

Anti-CD4 (OKT-4a)

Murine monoclonal antibodies reacting with CD4, which is solely located on T-helper lymphocytes and monocytes/macrophages, may also be suited for immunosuppression.

Campath-1H (Alemtuzumab)

This is a humanized anti-CD52 ►monoclonal antibody. At present it is in clinical use after bone marrow transplantation and for the treatment of refractory chronic lymphocytic leukemia.

Inhibitors of Interleukin-2-Induced T-Lymphocyte Proliferation

Anti-Interleukin-2 Receptor (CD25) Antibodies (Basiliximab/Daclizumab/Inolimomab)

The chimeric human/murine (basiliximab and daclizumab) or murine (inolimomab) monoclonal antibodies are specifically directed against a part (CD25) of the interleukin-2 (IL-2) receptor. Binding of one of these antibodies to CD25 thereby displaces physiological IL-2 and prevents proliferation of activated T-lymphocytes.

P70S6 Kinase Inhibitors (Sirolimus, Everolimus, Temsirolimus)

Sirolimus (SRL), also termed rapamycin is a macrolide lactone isolated from the ascomycete species *Streptomyces hygroscopicus*. After binding to its cytosolic receptor FKBP-12 the resulting complex inhibits the multifunctional serine–threonine kinase mTOR (mammalian target of rapamycin). Inhibition of mTOR prevents activation of the p70S6 kinase and successive

G₁ to S phase cell-cycle transition. Transition is required for the onset of IL-2 induced T-cell proliferation. Additionally, SRL also attenuates growth factor induced proliferation of several nonimmune cells and also inhibits metastatic tumor growth and angiogenesis.

Everolimus (RAD) is a synthetic derivative (40-O-(2-hydroxyethyl)rapamycin) of SRL with a shorter half-life. Its molecular mode of action resembles that of SRL.

Another SRL derivative, temsirolimus, is in clinical trials for the treatment of various leukemias.

Inhibitors of IL-2 Synthesis

► Glucocorticoids

After diffusion through the cell membrane, glucocorticoids bind to their specific receptor, which acts as hormone-dependent transcription factor. In the nucleus transcription of several genes is regulated after binding at specific glucocorticoid responsive elements of the DNA or by transactivation through interaction with other coactivators. In several cell types glucocorticoids induce transcription of the protein IκBα, which binds to the transcription factor NFκB thus preventing its activation and nuclear translocation. The important feature for the strong immunosuppressive action of glucocorticoids is inhibition of the synthesis of those cytokines that are involved in the activation of lymphocytes, i.e., interleukin-1 and -2. Therefore, especially cellular immune reactions are affected. In addition, a short-term reduction of circulating lymphocytes in the blood is observed after glucocorticoid treatment, due to a reversible sequestration in the bone marrow.

Calcineurin Phosphatase Inhibitors

Cyclosporine A

Cyclosporine A (CsA) is a water-insoluble cyclic peptide from a fungus composed of 11 amino acids. CsA binds to its cytosolic receptor cyclophilin. The CsA/cyclophilin complex reduces the activity of the protein phosphatase calcineurin. Inhibition of this enzyme activity interrupts antigen receptor-induced activation and translocation of the transcription factor ►NFAT to the nucleus which is essential for the induction of cytokine synthesis in T-lymphocytes.

Tacrolimus

Tacrolimus (TRL), in the past also named FK506, belongs to the group of macrolides and is produced by a special actinomycete species. TRL binds to its cytosolic receptor FKBP-12, however, it also blocks calcineurin activity and subsequently cytokine synthesis.

Pimecrolimus (ASM 981)

ASM 981 is an ascomycin derivative that has been approved for topical application only.

Inhibitors of Interaction between Antigen or Antigen-Presenting Cells and T-Lymphocytes

15-Deoxy-Sperguanilin (Gusperimus)

The exact molecular mode of action of this drug, which is so far only marketed in Japan, is not clear. Presumably, it interferes with antigen processing in APCs.

CTLA4-Ig Fusion Protein (Belatacept)

This is an immunoglobulin fusion protein with the cytotoxic lymphocyte antigen 4 (CTLA-4) receptor. By binding to CD80/86 on APCs it inhibits the CD28 costimulatory signal in lymphocytes. It is speculated that this can result in tolerance but up to now there is only experimental data [3, 4].

Anti-CD40 Specific Monoclonals

Such antibodies, which are still in an experimental status inhibit CD40–CD40L (=CD154) interaction.

Vitamin D3 Analogues

In addition to its classical role as regulator of calcium homeostasis, 1,25-dihydroxyvitamin D₃ (calcitriol) displays immunosuppressive properties. Inhibition of T-lymphocyte proliferation seems to be mediated via regulation of CD80/86 costimulatory molecule expression on APCs. For clinical use as immunosuppressant, however, analogues of vitamin D₃ that do not influence calcium metabolism are needed.

Inhibitors of Adhesion Molecules and Lymphocyte Homing

CD2 Antagonist (Alefacept)

Alefacept is a human recombinant integrin LFA3–IgG fusion protein that binds to the CD2 receptor thus blocking T-cell activation.

Anti-CD11a Antibodies (Antilfa, Efalizumab)

These are murine or humanized antileukocyte functional antigen (LFA)-1α antibodies that block LFA-1α (CD11a)–ICAM-1 (CD54) interaction.

Anti-ICAM-1 Antibody (Enlimomab)

This murine antibody also blocks LFA-1α–ICAM-1 interaction.

FTY720 (Fingolimod)

This experimental drug is a derivative of myriocin. After phosphorylation FTY720 modulates chemotactic responses and lymphocyte trafficking, leading to reversible lymphocyte sequestration in secondary lymphoid tissues. It is in clinical trials for the treatment of multiple sclerosis.

Clinical Use (Including Side Effects)

Specific immunity is a highly sophisticated defense mechanism of higher organisms. A high level of

specificity is given by immune responses directed against antigen epitopes of pathogenic microorganisms, foreign (transplant) or transformed cells (tumor), or even autologous cells (autoimmunity). Thus treatment with immunosuppressive agents is indicated for transplantations, systemic autoimmune diseases, chronic inflammatory diseases, and certain types of allergic diseases.

In any case, immunosuppressive therapy has to be carefully balanced in order to achieve a sufficient reduction of the unwanted immune reactions and to avoid complete suppression of host defense that might result in bacterial, viral, fungal, and parasitary infections or increases in the incidence of various malignancies. Besides these unspecific adverse effects of all immunosuppressants drug-specific side effects, i.e., organ toxicity or metabolic alterations (hypertension, hyperglycemia, hyperlipidemia) are apparent [5, 6]. All antiproliferative immunosuppressive drugs can lead to hematologic disorders like leukopenia, thrombocytopenia, and anemia indicating a continuous control of hematopoiesis.

Indications for the clinical use of immunosuppressive drugs are: transplantation, autoimmune diseases, chronic inflammatory diseases, allergic reactions.

Transplantation

The allogenic transplantation of solid organs, i.e., kidney, heart, liver, lung, small intestine, and pancreas, constitutes an absolute indication for a lifelong treatment with immunosuppressive agents. The major goal of the therapy is to prevent acute or chronic rejection of the donor organ. Patients transplanted with allogenic bone marrow have to be saved from undesired immune reactions caused by transferred mature lymphocytes (graft-versus-host reaction).

Immunosuppressive therapy in allogenic transplantation consists of an induction phase (first 4 weeks) followed by the maintenance phase (normally lifelong). Because of the higher risk of acute organ rejection especially in the induction phase a combination of several immunosuppressants including antibodies with high dosages and blood target concentrations are utilized.

Treatment with specific antibodies (ALG, ATG, anti-CD3, anti-CD25) is indicated during the induction phase after transplantation and in the case of acute rejection for short time periods. Therapy with nonhuman antibodies may cause sensitization. Muromonab-CD3 might initiate a cytokine release syndrome (fever, chills, headache).

During the maintenance phase dose reductions are aimed. However, in most cases a dual or triple combination therapy is still necessary. The use of drugs with different mechanisms of immunosuppressive action allows the application of lower doses additionally resulting in decreased toxicity.

Glucocorticoids belong to the oldest immunosuppressive agents given to transplant recipients and are

part of most treatment regimens. However, due to their numerous side effects (see: glucocorticoids) particularly at high and continuous dosage, they are withdrawn or doses are largely reduced within months.

Current immunosuppressive regimens depend on a combination of a T-lymphocyte specific calcineurin phosphatase inhibitor (CsA or TRL) and a newer antiproliferative drug (MPA, SRL, RAD). Although CsA and TRL display a similar mechanism of action some differences in their side effect profiles are obvious. The most common unwanted effects of CsA comprise of nephrotoxicity, hepatotoxicity, neurotoxicity, hypertension, hyperlipidemia, increased diabetogenic risk, hirsutism, and gingival hyperplasia. TRL appears equally nephrotoxic but hypertension and hyperlipidemia occurs less; hyperglycemia is more frequent than after CsA treatment. Gingival hyperplasia is lacking but TRL may result in alopecia. In contrast, MPA, SRL, and RAD are not nephrotoxic, however, myelosuppression is a common side effect of these drugs. The use of MPA is limited by dose-related gastrointestinal disorders. SRL, RAD, CsA, TRL, and glucocorticoids induce hyperlipidemic effects including increases in cholesterol as well as triglyceride serum levels that have to be treated with hydroxymethylglutaryl coenzyme A reductase inhibitors (►HMG-CoA-reductase-inhibitors).

Most immunosuppressive drugs applied in the maintenance phase after transplantation are substrates of the efflux pump P-glycoprotein (MDR1) and the cytochrome P450 3A4/A5 (CYP3A4/A5) metabolizing enzyme system in the gastrointestinal tract and the liver. Both MDR1 and CYP 3A4/A5 are focal points of numerous pharmacokinetic interactions. Thus, coadministered drugs can either induce (e.g., rifampicin, St John's wort) or inhibit (e.g., erythromycin, diltiazem, fluconazol) MDR1 and CYP3A4/A5 expression or activity resulting in reduced or elevated blood concentrations of the immunosuppressants. For this reason it is also advised that the oral intake of CsA and SRL should be interrupted by a 4 h interval.

Therapeutic Drug Monitoring

Individualization of treatment by therapeutic drug monitoring (TDM) is indicated for most immunosuppressive agents exhibiting a narrow therapeutic index and broad interindividual pharmacokinetic variability. A careful monitoring of the respective target concentrations of MPA, CsA, TRL, SRL, and RAD can help to minimize their toxicity and to reduce the incidence of acute organ rejection. It is a matter of discussion whether trough or maximum blood levels should be determined in clinical practice [7, 8].

Autoimmune Diseases

Immunosuppressive agents are indicated for the therapy of systemic autoimmune diseases (e.g., systemic lupus

erythematodes, myasthenia gravis) exhibiting severe organ-specific disorders which cannot be treated by other drugs. In general, immunosuppressive treatment of autoimmune disease is less successful as compared to organ transplantation, because of intraindividual variabilities.

Chronic Inflammatory Diseases

In the pathogenesis of many chronic inflammatory diseases (e.g., ►rheumatoid arthritis, glomerulonephritis, colitis ulcerosa, Morbus Crohn, atopic dermatitis, psoriasis) autoimmune processes play an important role, too. Although first of all nonsteroidal antiinflammatory agents or glucocorticoids should be applied, immunosuppressive agents may also be indicated.

Low-dose methotrexate is used for treatment of rheumatoid arthritis despite side effects such as disorders of the gastrointestinal tract and the liver. Leflunomide is also approved for this indication, however, hepatotoxicity limits its use as first option.

For the topical treatment of some chronic inflammatory skin diseases (like atopic dermatitis) immunosuppressive macrolides (like TRL and pimecrolimus) that permeate the inflamed epidermis are of benefit for patients. Severe side effects comparable to those after systemic application of TRL in transplanted patients (see above) have not been observed so far. For the treatment of psoriasis vulgaris these drugs are less effective. The CD2 antagonist alefacept may be a suitable alternative to allergic reactions.

Allergic reactions (especially those of type IV) can lead to disorders which resemble autoimmune or chronic inflammatory diseases. If an immediate elimination of the antigen is not feasible, immunosuppressive drugs can represent a reasonable addendum.

- Immune Defense
- Allergy
- Inflammation
- TOR Signalling
- Humanized Monoclonal Antibodies
- Glucocorticoids
- Non-steroidal Anti-inflammatory Drugs

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IMPDH

Inosine monophosphate dehydrogenase (IMPDH) is the key enzyme of purine nucleotide biosynthesis. Proliferation of activated lymphocytes depends on rapid de novo production of purine nucleotides for DNA synthesis.

- Immunosuppressive Agents

Importins

Importins are transport proteins at the nuclear pore complex, needed for the selective import of proteins into the nucleus. They recognize nuclear localization signal sequences of cargo proteins.

- Small GTPases

In Silico Biology

- Bioinformatics
- Molecular Modelling

Inclusion Cell Disease

Hereditary lysosomal storage disorder with an heterogeneous clinical presentation including mental retardation and skeletal changes. The disease is caused by

mutations in the gene encoding the (UDP)-*N*-acetylglucosamine:*N*-acetylglucosaminyl-1-phosphotransferase. This enzyme mediates normally the transfer of mannose-6-phosphate to precursor lysosomal enzymes. Due to the loss of enzyme function, lysosomal enzymes are secreted instead of being correctly targeted to the lysosomes. As a consequence, large amounts of acid mucopolysaccharides, sphingolipids, and/or glycolipids accumulate in the lysosomes.

►Protein Trafficking and Quality Control

Incretin Hormones

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Synonyms

Incretins: gut hormones that increase glucose-stimulated insulin secretion; GLP-1: glucagon-like peptide-1; GIP: Gastric inhibitory peptide or glucose-dependent insulinotropic peptide

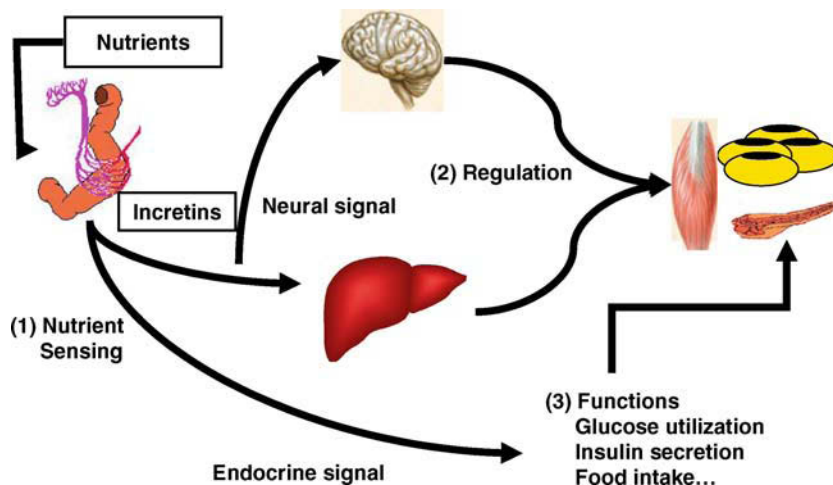
Definition

An oral glucose load increases insulin secretion more efficiently than when glucose is administered

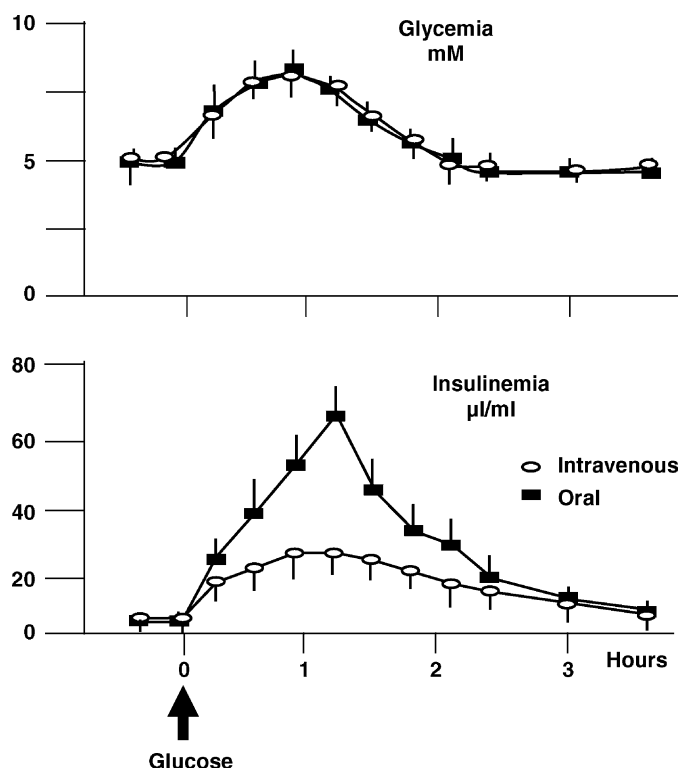
intravenously. This is due to the release by endocrine epithelial intestinal cells of hormone named incretins.

Basic Characteristics

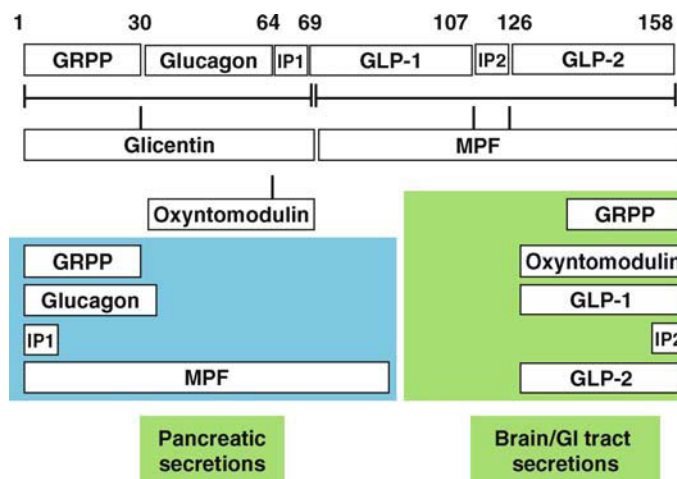
Energy homeostasis is finely tuned by neural and endocrine factors that equilibrate energy intake and expenditure. An impairment of this balance would lead to metabolic diseases such as obesity, diabetes, ►**lipodistrophy**, or cachexia. To trigger metabolic programs suitable to maintain energy homeostasis, the body should first quantitatively and qualitatively detect nutrients; this is called metabolic reflex (Fig. 1). It is the role of specialized cells from the enteric area, known as the intestinal epithelium, where most of the nutrients are absorbed. These cells send a signal of endocrine or neural origin that triggers functions aiming to address energy to the proper tissues for storage or utilization. Among the signals involved in the metabolic reflex are incretins. The hormones released from the gut in response to nutrient ingestion potentiate glucose-stimulated insulin secretion. Indeed, the administration of an oral glucose load leads to a much greater stimulation of insulin release from the pancreatic β -cells of the Langerhans islet, compared with a similar glucose challenge given intravenously (Fig. 2). This entero-insular axis (Corresponds to the physical and biochemical connections between the gut and the pancreatic cells from the Langerhans islets. These connections are mostly ensured by the autonomic nervous system and hormones secreted by the gut, which directly targets the pancreas or needs the brain as a relay.) was responsible for ~50–70% of the postprandial insulin release [1]. From humoral extract of intestinal origin a first 42 amino acid long glucose-dependent ►**insulinotropic** peptide



Incretin Hormones. Figure 1 The metabolic reflex. Nutrients, essentially glucose, upon absorption will trigger the secretion of intestinal signals such as the incretins and be detected by specialized cells of neuronal origin and located in the hepatportal vein. The neural signal will be sent to the brain and together with the endocrine signal functions, it will be recruited.



Incretin Hormones. Figure 2 The incretin effect. Patients underwent an oral or equivalent intravenous glucose load so that the glycemic profiles became similar. In such conditions, the data show that the oral glucose load is more potent to stimulate insulin secretion than an equivalent intravenous glucose stimulus.



Incretin Hormones. Figure 3 Processing of the proglucagon. The proglucagon peptide is synthesized in pancreatic cells and cells from the gastrointestinal (GI) tract and the brain. Different proconvertases process the peptide so that in the pancreas the glucagon is produced whereas in the GI tract and the brain, the GLP-1 and GLP-2 peptides are mainly released.

(GIP) was isolated. It was characterized as being released by the duodenum following an oral glucose load and increasing glucose-stimulated insulin secretion. However, this peptide was not able to account fully for the

incretin effect. In 1983, Bell and coworkers cloned the proglucagon gene and identified two novel peptides, close to the pancreatic glucagon structure named glucagon-like peptide (GLP) one and two (Fig. 3). The first is a

37 amino acid long peptide but inactive on insulin secretion GLP-1. The second GLP-2, of similar length regulates epithelial intestinal growth. Later on, in 1986, the 7–37 amino acids fraction of the GLP-1 was discovered as the active form of the peptide on glucose-stimulated insulin secretion (GLP-1_{7–37} or 7–36amide). Then experiments of ►immunoneutralization, using mice deleted for the GIP and GLP-1 receptors, demonstrated that both peptides are dominantly involved in nutrient-stimulated insulin secretion and fully account for the incretin effect. GIP and GLP-1 are synthesized as propeptide. This is a common characteristic of peptide hormone that requires an intermediate form based on a longer peptide which will be processed during maturation and exocytosis by endo- and ►exopeptidases named proconvertases (PC_{1/3-2}, ►furin). The proglucagon gene is expressed in the α pancreatic, the intestinal epithelial, and some neuronal cells in the brain. Therefore, different sets of PC are involved in allowing glucagon to be secreted by the pancreas and GLP-1 by the intestine and brain cells.

Glucose-stimulated insulin secretion is the physiological action of incretins which has been the most described [2]. The receptors of both incretins are present at the surface of the insulin-secreting pancreatic B cells. Upon binding of the corresponding hormone, the cAMP concentration increases and it activates a protein kinase A and thereby, regulates numerous cellular functions involved in the closing of the ATP-sensitive K⁺ channel, calcium entry, and vesicular trafficking for the exocytosis of insulin-containing granules [3]. As insulin secretion depends on the energy status of the cell, i.e., the ATP/ADP ratio, the remarkable property of incretins is to increase insulin secretion in response to an earlier or a simultaneous priming of β -cell energy metabolism by hyperglycemia. Consequently, excessive amount of incretins cannot increase insulin secretion in euglycemic condition, such as while fasting, and therefore, cannot induce deleterious hypoglycemia. These hormones are totally dedicated to the absorptive phase and are hence, considered messengers of energy intake. Both hormones are equipotent in terms of insulin secretion. However, the GIP receptor is largely distributed at the surface of the β cells, the adipose tissue, the stomach, the bone, the endothelial cells, the lung, the liver, the brain, the heart, and the gut whereas the GLP-1 receptor is restricted to the brain, the β cells, and the heart.

Importantly, both incretins when secreted by the intestine are rapidly degraded by the ►dipeptidyl peptidase IV (DPPIV), which removed the two amino-terminus histidine-alanine residues, thereby, inactivating the incretins. This enzyme is present at the surface of the epithelial intestinal cells and capillaries in the vicinity of the K and L cells secreting GIP and GLP-1, respectively. It is also present in the

endothelium and degrades continuously the incretins. The DPPIV limits the half-life of the active forms of the peptides to less than a minute in the hepatoportal and systemic blood and hence, the accessibility of the β cell to the peptides. K cells are located in the duodenum, i.e., where most of the glucose ingested is absorbed and upon intestinal glucose absorption a large amount of GIP is secreted within minutes. Conversely, the L cells are distal to the glucose absorption location, in the proximal colon. The very short half-life and the distal location of the L cells suggest that GLP-1 secretion and action depend on an intermediary mechanism. This relay could be the enteric-hepato-portal glucose sensing system. This mechanism involves nutrients, upon intestinal absorption, accumulating into the hepatoportal vein and activating the corresponding glucose sensitive cells. The latter send a signal to the brain for the control of GLP-1 secretion and peripheral functions. This signal is of neural origin as vagotomy prevents glucose-stimulated GLP-1 secretion. Similarly, the control of insulin secretion does not wholly depend on the binding of GLP-1 on β cells. Numerous recent evidences show that a neural relay depending on GLP-1 is required to fully account for oral glucose-stimulated insulin secretion. The hepatoportal infusion of the GLP-1 receptor antagonist prevents oral glucose-induced insulin secretion and similar observations were made in mice detected for the GLP-1 receptor. In addition to the role of GLP-1 on the enteric-hepato-portal area for the control of insulin secretion, brain GLP-1 signaling is required for the control of the endocrine pancreatic function. Upon oral glucose absorption, in addition to the enteric secretion of GLP-1, arguments suggest that GLP-1 is also released by the brain. Areas of the brain stem are connected to the enteric areas with axons originating from the vagus nerve. The ascendant branch transmits the glucose signals and triggers cells in the brain stem. The proglucagon gene is expressed in cells from the area postrema and the nucleus from the solitary tract. Furthermore, neurons from both areas send projections to nuclei of the hypothalamus. As a matter of fact, cells in the hypothalamic areas express the GLP-1 receptor. Since the hypothalamus is now considered a major player responsible for the management of energy metabolism, brain GLP-1 signaling would represent an important contributor. The blockage of brain GLP-1 signaling by Exendin 9, an antagonist of the GLP-1 receptor, prevents oral glucose-stimulated insulin secretion showing hence, the major role of brain GLP-1 signaling. Interestingly, no role of GIP has been described on the enteric-hepato-portal glucose sensor (Corresponds to a structure present in the hepatoportal vein, which is located between the intestine and the liver and collects all mesenteric blood. Cells sensitive to glucose has been suggested to be located in the

hepatoportal vein.), suggesting that GIP is produced in larger amount (nM vs. pM, concentration in the blood of GIP and GLP-1, respectively) than GLP-1. GIP could directly reach the β cells and trigger insulin secretion whereas this seems unlikely for GLP-1 as the concentration is too low despite the very high affinity of the receptor.

The effect of incretins is not restricted to insulin secretion. GIP is also responsible, to some extent, to lipogenesis. GLP-1 has a positive isotropic effect on the heart rate, reducing gastric emptying, glucagon secretion, and food intake. Furthermore, GLP-1 also favors β -cell growth. All the latter effects are considered antidiabetic and antiobesity. Hence, numerous therapeutic strategies have been generated based on incretin secretion. However, the actors preferred strategies based on GLP-1 as the peptide conserves its effectiveness during diabetes, whereas, GIP does not seem to be potent anymore. Therefore, several strategies have been proposed based on GLP-1 physiology. The first one involves the GLP-1 analogue Exendin μ , discovered from the saliva of the Gila monster. This peptide presents a 60–70% homology to GLP-1, binds to the receptor with a high affinity, and is totally insensitive to DPP-IV. Furthermore, the renal clearance of Exendin μ is low, extending the half-life of the peptide to 12–18 h. The clinical trials showed an important reduction of the HbA1c, an index to the extent of diabetes. However, one to two subcutaneous injections per day are necessary for a full effectiveness of the therapy. Several strategies for further increasing its half life have been proposed based on microencapsulation of the peptide into slow-diffusing gels. Hence, a 1-week to a 1-month injection is proposed. Furthermore, there was also a significant reduction of the body weight which was probably due to the satiety effect related to the lowering of gastric emptying of the peptide. A second strategy involves the inhibition of DPP-IV activity. Although not specific to GLP-1, clinical trials showed an increased glucose-stimulated insulin secretion and a reduced HbA1c. This strategy has the physiological advantage to increase the secretion of GLP-1, i.e., at its enteric location, where important effects on insulin secretion have been showed. This strategy clearly represents a physiological approach, however, its lack of specificity could mask some secondary deleterious or unexpected side effects. The third strategy involves the physiological secretion of GLP-1 by means of dietary fibers. Recent evidence showed that the weight and blood-glucose concentration were reduced when patients or diabetic animals were eating dietary fibers such as oligofructose. The mechanisms involved the GLP-1 receptor as well as an increased expression of the proglucagon gene and concentration of the corresponding peptide in the intestine. Furthermore, the number of

L cells was augmented. This latter strategy is also promising as dietary fibers trigger a physiological secretion of the peptide in the vicinity of glucose sensitive cells, inducing the whole physiological cascade of action leading to the control of energy homeostasis.

In conclusion, the discovery of incretins has now come to an end in terms of therapeutic strategy of metabolic diseases since new medicines will be put on the market these coming years. However, a lot more needs to be done with regard to the physiological role of the hormones. Whereas the major filed of investigation for GLP-1 now relates to the effect of the peptide on the central nervous system and on β -cell proliferation, for GIP it remains to understand its role in the numerous tissues where the corresponding receptor is expressed.

Drugs

The new drugs that are derived from incretin studies are currently getting authorization to be put on the market. The first one are GLP-1 like molecules. Exenatide (Amylin Corp.) is a peptide issued from the saliva of the Gila monster, a lizard producing large quantities of this peptide when eating its prey. This peptide binds the GLP-1 receptor with an affinity similar to GLP-1 but is not a substrate of DPP-IV and is hence, much stable. Exendin-derived peptides are currently being made and new formulation should ensure a 1 week-long stability allowing, for the diabetic patient, a once-a-week convenient injection. GLP-1 analogues are also being generated. These peptides have been so designed that the alanine in position 2 of the peptide has been removed to avoid the degradation of the molecule by the DPP-IV. Further modifications allow the analogue to bind to albumin and further increase its half life. Another set of strategies consists in inhibiting DPP-IV activity. The advantage of such strategy is that the compound can be administered orally whereas the peptides have to be injected subcutaneously. Both strategies should be complementary to improve the blood glucose profiles of the diabetic patients.

► Diabetes Mellitus

► Appetite Control

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Inducible (Immunological) Nitric Oxide Synthase (iNOS)

Induced by bacterial lipopolysaccharides or immune cytokines in macrophages, smooth muscle cells, and glia cells. Ca^{2+} is not required for the enzyme activation.

► Nitric Oxide

Inflammation

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Definition

Inflammation occurs when a living tissue is injured or infected by microorganisms. It is a beneficial, self-limited response that requires phagocytic cells and elements of circulating plasma to enter the affected area. In principle it may achieve resolution and repair as the ideal outcome of inflammation. The persistent accumulation and activation of leukocytes is a hallmark of chronic inflammation.

Basic Mechanisms

Introduction

The clinical characteristics of acute inflammation are familiar to anyone who has suffered from a burned or infected finger. The account comprises the cardinal symptoms of inflammation with heat, redness, and painful swelling that were reported by the Roman encyclopedist Celsus as calor, rubor, tumor, and dolor. Galen of Pergamon added a further sign of inflammation, the impaired function or *functio laesa*. Though unpleasant, these signs are indicators of useful processes going on with the aim for limiting tissue damage and infection and initiating repair. Inflammation starts after an initial injury by mechanical trauma, infections, UV-irradiation, burns, ischemia, and many others. It initially comprises the release of chemical mediators like histamine from a population of cells that are distributed throughout the connective tissue and are extremely sensitive to injury, the mast cells. The same is true for the blood basophil, which in many aspects resembles the mast cell. Histamine and secondary

mediators like eicosanoids and nitric oxide cause vasodilatation and increase the calibre of arterioles, capillaries, and venules, which results in increased blood flow to the injured area and consequently redness and heat. An increase in the vascular permeability causes the loss of solutes and proteins from the blood plasma, a process called exsudation, which leads to swelling and oedema formation (tumor). The local increase in tissue turgor and the activation of the kinin cascade with the generation of particularly bradykinin are major factors causing pain, the fourth cardinal symptom of inflammation. Besides these changes of the microcirculation the acute inflammatory response essentially requires the extravasation of leukocytes and phagocytosis of microorganism and cell or tissue debris.

Cells and Mediators of Acute Inflammation

The migration of phagocytic cells to the site of damage is one of the most fundamental components of the acute inflammatory response, and the key players in this process will be presented next.

The Endothelial Cell

The vascular endothelium plays an important role in regulation of vascular tone and permeability. Dilatation of arterioles to increase blood flow and constriction of endothelial cells of postcapillary venules causing exsudation of plasma constituents illustrates the complex nature of this cell type. Moreover, by expression of adhesion molecules and secretion of ►chemokines endothelial cells play an important role in the recruitment of leukocytes to the inflamed area. Endothelial cells express two basic types of adhesion molecules on their surface:

1. ►Selectins (E-Selectin, P-Selectin)
2. Members of the immunoglobulin gene superfamily (VCAM-1, ICAM-1, ICAM-2)

Selectins are a family of glycoproteins that allow the initial attachment and rolling of leukocytes on endothelial cells. Selectins are not expressed on the surface of resting endothelial cells but are exposed upon activation with a number of mediators like ►interleukin-1 (IL-1), tumor necrosis factor α (►TNF α), lipopolysaccharide or thrombin. Two endothelial selectins have been reported. E-selectin is synthesized de novo and expressed on endothelial cell surface following stimulation. The specific ligand for E-selectin is stored in Weibel–Palade bodies of endothelial cells and in α -granules of platelets and is translocated within minutes to the plasma membrane following exposure to inflammatory mediators. The ligand for P-selectin is the so-called P-selectin glycoprotein ligand-1 (PSGL-1), which has the sugar lacto-n-fucopentose III in its core domain and is present on hematopoietic cells.

The second class of adhesion molecules expressed on endothelial cells belong to the immunoglobulin gene superfamily. These transmembrane glycoproteins structurally resemble in certain parts the structure of immunoglobulins. Whereas intercellular cell adhesion molecule-1 (ICAM-1) and ICAM-2 are constitutively expressed on endothelial cells, the third member vascular cell adhesion molecule-1 (VCAM-1) is not present on resting endothelial cells but is upregulated together with ICAM-1 in the course of acute inflammation by cytokines like IL-1 or TNF α within a few hours. The counterreceptor on the cell membrane of leukocytes that binds to ICAMs and VCAM-1 belong to the ► **integrin** family of adhesion molecules.

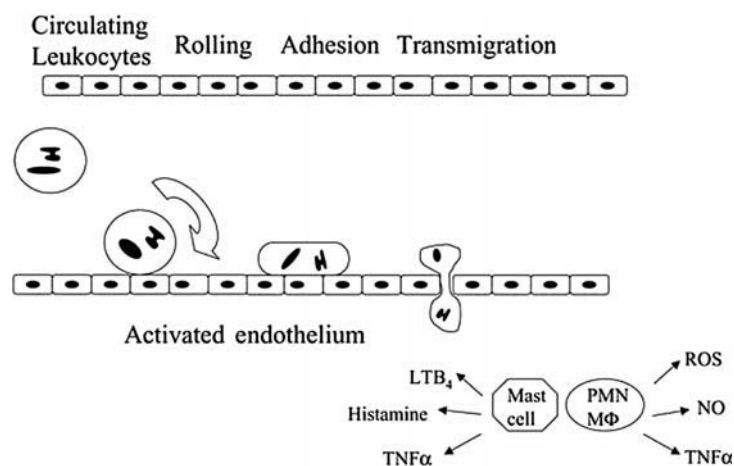
The Neutrophil

In the very early phases of the acute inflammatory response most of the cells invading the damaged area are polymorphonuclear neutrophils, also denoted as PMNs, which serve as initial line of defense and source of proinflammatory cytokines. These cells, which usually live for 4–5 days, circulate in the blood until they are attracted by ► **chemokines** into injured tissues. Whereas physical injury does not recruit many neutrophils, infections with bacteria or fungi elicit a striking neutrophil response. The characteristic pus of a bacterial abscess is composed mainly of ► **apoptotic** (*apoptosis*) and necrotic PMNs. Emigration of neutrophils from the blood starts with a process denoted as **margination** where neutrophils come to lie at the periphery of flowing blood cells and adhere to endothelial cells (**Fig. 1**). L-Selectin is expressed

constitutively on most leukocytes and interacts with ligands that are induced in endothelial cells exposed to inflammatory cytokines. The second class of surface molecules that mediate adhesion of neutrophils and monocytes to endothelial cells are the **integrins**. Leukocyte integrins are heterodimeric transmembrane proteins consisting of α and β chains with short cytoplasmic domains that are engaged in signal transduction. Many cells express integrins, and some of the known integrins are cell-type specific. Integrins mediate firm adhesion that follows the initial rolling of leukocytes along the endothelial cell lining. All the leukocyte integrins share a common small subunit, the β_2 subunit. There are at least four larger α subunits known that can associate with the β_2 subunit to form unique receptors. It is these β_2 -integrins that adhere to the endothelial ICAMs in the course of acute inflammation. Once the neutrophils have made firm contact to the endothelial cells they protrude pseudopodia and leave the blood vessel by squeezing through the gap between adjacent endothelial cells and subsequently pass the basement membrane (**Fig. 1**). The whole process takes a few minutes.

The Monocyte/Macrophage

The very early peak of neutrophil invasion into an inflamed area is followed several hours later by a wave of a second class of phagocytic cells, the macrophages. This biphasic pattern of inflammatory cell movement and accumulation is observed in most acute inflammatory responses. The mononuclear phagocyte in the blood is known as the monocyte and differentiates



Inflammation. Figure 1 Sequence of events in the recruitment of leukocytes in postcapillary venules adjacent to injured tissue. At the site of lesion, diverse reactive substances stimulate the endothelium to produce inflammatory cytokines, chemoattractants and other inflammatory mediators. The cytokine-activated endothelium expresses adhesion molecules that lead to the low affinity interactions between leukocytes and endothelium, which is mediated by selectins and described as rolling. Subsequently integrins mediate the firm adhesion of leukocytes, which allows emigration of the cells from venules into the interstitial compartment. Activated mast cells, PMNs and macrophages secrete cytokines (TNF α), lipid mediators (LTB $_4$) and other inflammatory players (histamine, NO).

into the macrophage upon entering into tissues. The differentiation process markedly increases the phagocytic and secretory capacities of the cell. Chemotactic factors that act on macrophages include complement cleavage products, membrane components of microorganisms and fibrin degradation products that also attract neutrophils. In addition, there are specific chemokines that act exclusively on macrophages. Macrophages in turn release large amounts of growth factors and cytokines.

Mediators of Inflammation

Many low weight compounds produced by microorganism-like formylated peptides as well as endogenous mediators are chemotactic for leukocytes and promote the inflammatory process. The main endogenous compounds are listed in Table 1 and are derived from activated plasma protein cascades that function as amplification mechanisms, are performed and released from activated cells or are de novo synthesized on demand by cells participating in or being affected by inflammatory events. The major modulators of leukocyte adhesion to endothelial cells are listed in Table 2.

Healing

The objectives of the inflammatory response can be viewed as a hierarchical ordered panel of events. The most successful consequence of an inflammatory response is the complete restoration of function and structure of the affected tissue, also denoted as resolution. If this is not possible, inflammation aims for healing by repair and replacement of lost tissue by scar tissue.

Central to resolution is the cessation of initiating stimuli, e.g. the killing of invading bacteria and

microorganisms, and the complete removal of inflammatory exudate. Whereas neutrophils carry out the killing of invading microorganisms that subsequently die by apoptosis, macrophages are mainly responsible for clearing dead neutrophils and fluid phase debris by phagocytosis and extremely active pinocytosis. It should be emphasized that the clearing of apoptotic cells by phagocytes is extremely fast and efficient, and dying cells release chemokines to speed up their removal by attracting macrophages. Moreover, cyto-

Inflammation. Table 1 Endogenously produced mediators of inflammation

Category	Mediators
Preformed mediators released from activated cells	Histamine
	Serotonin
	Lysosomal enzymes
Mediators derived from activated plasma protein cascades	Complement system
	Kinin cascade
	Clotting pathway
	Fibrinolytic system
De novo synthesized mediators	Prostaglandins
	Leukotrienes
	Cytokines
	Reactive oxygen species
	Nitric oxide

Inflammation. Table 2 Modulation of leukocyte adhesion to endothelial cells

Target cell	Endothelial cell	Leukocyte
Antiadhesive	Interleukin-4	Interleukin-4
	Interleukin-10	Interleukin-10
	Interleukin-13	Interleukin-13
		Prostacyclin
	Adenosine	Adenosine
	Nitric oxide (NO) glucocorticoids	Nitric oxide (NO)
Proadhesive	Tumor necrosis factor α (TNF α)	Tumor necrosis factor α (TNF α)
	Interleukin-1	Interleukin-1
	Interferon γ	Interferon γ
		Interleukin-8
		MCP-1
	Leukotrien B ₄	Leukotrien B ₄
	PAF	PAF
	Endotoxin	Endotoxin C3b, C5a
	Histamine	Neuropeptides

kines like interleukin-6 and antiinflammatory lipid mediators such as lipoxins or prostaglandins D₂ and E₂ play important roles in providing stop signals for acute inflammatory processes. Most macrophages emigrate from the inflamed site to reach draining lymph nodes. Subsequently tissue that has been lost must be replaced in an orderly fashion. The replacement of lost cells by compensatory proliferation and phenotypic change of surviving resident cells is part of the healing process. If the removal of inflammatory exudate fails, a process denoted as organization is triggered. The exudate is invaded by macrophages and fibroblasts and the formation of new blood vessels (angiogenesis) is initiated. This series of events finally results in the formation of tissue scar, which may be considered as an essential component of wound healing not only in skin.

Chronic Inflammation

If the endogenous control mechanisms of inflammation fail and resolution or healing by repair cannot be achieved, the inflammatory process may persist for weeks, months or even years and is termed chronic. The inflammatory macrophage is not only a ringmaster for safe resolution and repair of inflammation but also for chronicity of the disease. Some of the products secreted by macrophages are relevant to chronic inflammation such as IL-1, TNF α , IL-6 or IL-10, just to name a few. A further characteristic feature is the presence of activated B and T lymphocytes, which represent a local immune response to antigens presented to them by macrophages or dendritic cells. B cells differentiate upon activation to plasma cells that release immunoglobulins which in most cases is a good indicator of chronicity of inflammation. Further attempts to repair are clearly unsuccessful and tissue necrosis and inflammation continue as is the case in chronic ulcerative colitis or chronic pyelonephritis and other diseases. The mechanism by which the inflammatory response resolves is under intense investigations and may provide new targets in the treatment of chronic inflammation.

► Immune Defense

► Allergy

► Tumor Necrosis Factor (TNF)

► Non-steroidal Anti-inflammatory Drugs

► Glucocorticoids

► Immunosuppressive Agents

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Inflammatory Caspases

Inflammatory caspases (caspase-1, -4, -5, -11 and -12) constitute a subgroup of the caspase family. Caspase-1 is the best characterized member and is responsible for the proteolytic maturation and release of the pro-inflammatory cytokines pro-interleukin (IL)-1 β and pro-IL-18. Caspase-1 gets activated in inflammasome complexes upon cellular stress, cellular damage and infection.

► Caspases

Inflammatory Disorders

Inflammatory disorders are due to hyperactivity of leukocytes and overexpression of their associated integrins, cytokines, and chemokines, which leads to various disorders including arthritis, bowel diseases and other chronic inflammations.

► Inflammation

► Antiintegrins, Therapeutic and Diagnostic Implications

Influenza

Influenza is an acute viral disease caused by Influenza A (sporadic, epidemic, and pandemic) or B (sporadic outbreaks) virus. Symptoms typically occur suddenly and include high fever, chills, headache, muscle aches, sore throat, and malaise. Serious complications can be caused by bacterial superinfection of the respiratory tract.

► Antiviral Drugs

Infrared Spectroscopy

Infrared spectroscopy is an analytical technique which differentiates molecules on the basis of their vibrational states.

Innate Immunity

Innate immunity describes inborn effector mechanisms defending the organism against harmful foreign substances by humoral (►defensins, ►complement) or cellular (phagocytic cells, natural killer cells) mechanisms. In innate immunity, recognition of foreign substances is always by receptors such as pattern recognition receptors, which are germ-line configured and not rearranged like the T- and B-cell antigen receptors. From a phylogenetical point of view, the innate defense system developed much earlier than the adaptive immune system of lymphocytes; the innate system is found in many invertebrates.

- Immune Defense
- Toll-like Receptor

Inorganic Phosphate Transporters

Type 1 Na^+ -dependent inorganic phosphate transporters (NaPi-1) are a family of proteins isolated in a screen for genes that increase Na^+ -dependent inorganic phosphate transport. Further characterization with heterologous expression of the rat kidney NaPi-1 isoform in particular, demonstrated that phosphate transport did not correlate with the level of messenger RNA (mRNA) expressed. However, expression did correlate with the saturable transport of inorganic anions such as penicillin and probenecid. It has also been shown that NaPi-1 mediates an anion conductance that can be blocked by transport substrates. The sialic acid transporter sialin, and the vesicular glutamate transporters VGLUT1 and VGLUT2 are closely related to the type I Na^+ -dependent inorganic phosphate transporters.

- Vesicular Transporters

iNOS

- Inducible (immunological) Nitric Oxide Synthase
- Nitric Oxide

Inositol

D-myo-inositol is a six carbon polyalcohol in a ring structure arranged in a chair configuration.

- Phospholipid Kinases

Inositol 1,4,5-trisphosphate Receptors

- IP_3 Receptors
- Phospholipases

Insomnia

Insomnia is the condition of poor sleep. Primary insomnia is defined in the Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV) as “difficulty in initiating, maintaining or non-restorative sleep that results in clinically significant distress or impairment in social, occupational, or other important areas of functioning”. Secondary insomnia is brought about as a result of a separate pathological condition, for example depression, anxiety, schizophrenia or Parkinson's disease. Clinically, insomnia can be treated by attempting to improve the sufferers sleep hygiene (i.e. habits related to sleeping), or pharmacologically with ►hypnotic drugs.

- Sleep

Insulin

- Insulin Receptor
- Antidiabetic Drugs other than Insulin

Insulin Desensitization

Loss of the cell's responsiveness to the hormone insulin caused by pathological alterations in the insulin receptor signal transduction pathway, and often leading

to insulin resistance. Insulin desensitization is a common feature of the ►metabolic syndrome.

- Insulin Receptor
- Diabetes Mellitus

Insulin Receptor

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Synonyms

INSR (gene name)

Definition

The insulin receptor is a transmembrane ►receptor tyrosine kinase located in the plasma membrane of insulin-sensitive cells (e.g., adipocytes, myocytes, hepatocytes). It mediates the effect of insulin on specific cellular responses (e.g., glucose transport, glycogen synthesis, lipid synthesis, protein synthesis).

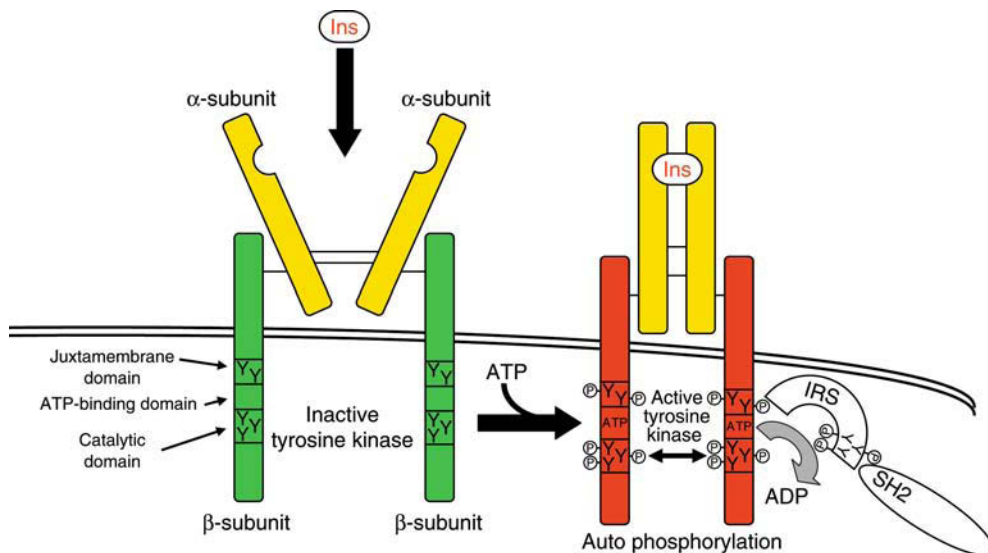
Basic Characteristics Structure and Function

The INSR is a heterotetrameric protein consisting of two extracellular α -subunits (molecular weight 135 kDa) and two membrane-spanning β -subunits (molecular weight 95 kDa). These subunits are covalently linked by disulfide bonds ($\alpha_2\beta_2$ -structure) (Fig. 1). The receptor is encoded by a single gene which is located on human chromosome 19 and consists of 22 exons (exon 1–11 = α -subunit, exon 12–22 = β -subunit). The single chain polypeptide precursor is posttranslationally cleaved in the endoplasmic reticulum into the α - and β -subunits which subsequently dimerize. Both subunits are glycosylated in the Golgi apparatus.

In addition to insulin, the INSR can also bind ►insulin-like growth factors (IGF1 and IGF2) albeit with considerably lower affinity (2 and 3 orders of magnitude, respectively).

The insulin-binding domain of the INSR is located within a cysteine-rich region of the α -subunits. Alternative splicing of exon 11 generates two isoforms of the α -subunit which differ in their C-terminus and in their tissue distribution (type A: leukocytes; type B: liver; type A and B: skeletal muscle and fat). The isoforms differ in their affinity to insulin (A > B), but their relevance for normal and impaired insulin action is not entirely clear [1, 2].

Activation of the tyrosine kinase activity of the INSR is essential for the receptor function. The tyrosine kinase domain of the INSR is localized in the



Insulin Receptor. Figure 1 Structure and function of the insulin receptor. Binding of insulin to the α -subunits (yellow) leads to activation of the intracellular tyrosine kinase (β -subunit) by autophosphorylation. The insulin receptor substrates (IRS) bind via a phospho-tyrosine binding domain to phosphorylated tyrosine residues in the juxtamembrane domain of the β -subunit. The receptor tyrosine kinase then phosphorylates specific tyrosine motifs (YMXM) within the IRS. These tyrosine phosphorylated motifs serve as docking sites for some adaptor proteins with SRC homology 2 (SH2) domains like the regulatory subunit of PI 3-kinase.

cytoplasmic region of the β -subunit. In the absence of insulin, the α -subunits strongly repress the tyrosine kinase activity of the β -subunits. Binding of insulin releases this block through a conformational change, and induces dimerization and/or oligomerization of β -subunits which results in receptor transautophosphorylation. While several phosphorylated tyrosine residues in the catalytic domain (Y1158, Y1162, Y1163 of human INSR) are essential for the kinase activity of the receptor, phosphorylation of two tyrosine residues in the juxtamembrane domain (Y965 and Y975 of human INSR) is critical for the interaction of the receptor with other signaling components of the insulin receptor signaling cascade (Fig. 1) [1, 3].

Signaling through the INSR systems has evolved early and has been highly conserved during evolution. Proteins related to the human INSR gene product have been found in *Caenorhabditis elegans* (DAF-2) as well as in *Drosophila melanogaster* (DIR). Insulin-like signaling peptides (e.g., bombyxin) have been found in such distantly related organisms as the silkworm [1].

Structurally and functionally related receptors to the INSR in mammalian organisms are the insulin-like growth factor 1 receptor (IGF1R) and the insulin receptor-related receptor (INSRR). Although the function and signal transduction of the IGF1R and the INSRR resembles that of the INSR, major differences appear in the tissue distribution of the receptors. In contrast to the INSR, the IGF1R is expressed in adipocytes only at low levels and is almost absent in hepatocytes. The INSRR is an orphan receptor whose endogenous ligand is yet unknown. It has been found in few neuronal cell types (e.g., dorsal root ganglion and trigeminal neurons) and in the kidney.

Deletion of the INSR gene results in normal development but early postnatal lethality because of ketoacidosis. Organ specific INSR-knockout models (>95% reduction of the receptor protein content in the organ) exhibit less severe phenotypes. Mice with a liver-specific insulin receptor knockout (LIRKO) showed severe insulin resistance and hyperglycemia due to an increased hepatic **gluconeogenesis**, whereas the tissue-specific knockout of the insulin receptor in pancreatic β -cells (β -IRKO) results in an altered insulin secretion comparable to that seen in type-2-diabetes. Specific knockout of the IGF1R in β -cells results in a similar phenotype. In contrast, mice with a skeletal muscle-specific insulin receptor knockout (MIRKO) had normal blood glucose and normal glucose tolerance but elevated serum free fatty acids and triglycerides. Interestingly, mice selectively lacking the insulin receptor in adipose tissue (FIRKO) were protected against obesity and obesity-related glucose intolerance and showed an extended lifespan. Mice with a neuron-specific INSR knockout (NIRKO) developed an obese phenotype with mild insulin resistance and impaired

fertility. These central effects of insulin on the peripheral energy metabolism reflect an important role of insulin in the regulation of neuropeptide expression (e.g., NPY, POMC) in the brain. Combined deletion of the INSR, IGF1R, and the INSRR in male (XY) mice results in a female phenotype with ovaries, thereby providing evidence that the insulin receptor tyrosine kinase family is essential for male sex differentiation.

Taken together, these data emphasize the importance of insulin action in the liver for glucose homeostasis, in the regulation of insulin secretion from β -cells and indicate that the INSR plays an important role in the central regulation of body weight and reproduction [4, 5].

There are two clinical syndromes with an impaired function of the INSR: Leprechaunism is a rare genetic disease characterized by growth retardation, hyperinsulinemia, and insulin resistance due to mutations in the INSR gene. Acanthosis nigricans is a syndrome of hyperpigmentation and hyperandrogenism associated with hyperinsulinemia and **diabetes mellitus**. Insulin resistance in this syndrome is either due to mutations in the insulin receptor gene (type A), or to autoantibodies to the INSR (type B). Some patients with insulin resistance due to decreased expression, increased degradation or impaired insulin binding of the INSR have been described.

Signal Transduction and Insulin Action

Stimulation of the insulin receptor results in the activation of two major pathways [3]: (i) the **mitogen-activated protein (MAP) kinase cascade** (discussed in chapter **MAP kinase cascade**) and (ii) the **phosphatidylinositol 3-kinase (PI 3-kinase)** pathway which has been extensively studied in the context of the metabolic responses to insulin (summarized in Table 1 and Fig. 2).

The major intracellular target molecules of the tyrosine kinase activity of the INSR β -subunit are the insulin receptor substrates (IRS). Interaction of phosphotyrosine-binding domains (PTB) within the N-terminal region of an IRS with the juxtamembrane phosphotyrosines of the INSR β -subunit results in tyrosine phosphorylation of consensus motifs (YMXM) in the C-terminus of IRS (Fig. 1). Four different mammalian IRS isoforms have been identified (IRS1-4). IRS1 knockout mice develop a mild state of insulin resistance (impaired glucose intolerance) without diabetes, whereas knockout of the IRS2 gene causes a phenotype with severe insulin resistance (liver > muscle), diabetes, and impaired pancreatic β -cell function. Both IRS1 and IRS2 knockout mice exhibit growth retardation (IRS1 > IRS2). IRS3 knockout mice appear to be normal, whereas disruption of the IRS4 gene produces mild glucose intolerance and growth retardation in male animals. IRS1 and IRS2 are ubiquitously expressed. IRS3 is expressed only in

Insulin Receptor. Table 1 The effect of insulin on energy and glucose homeostasis

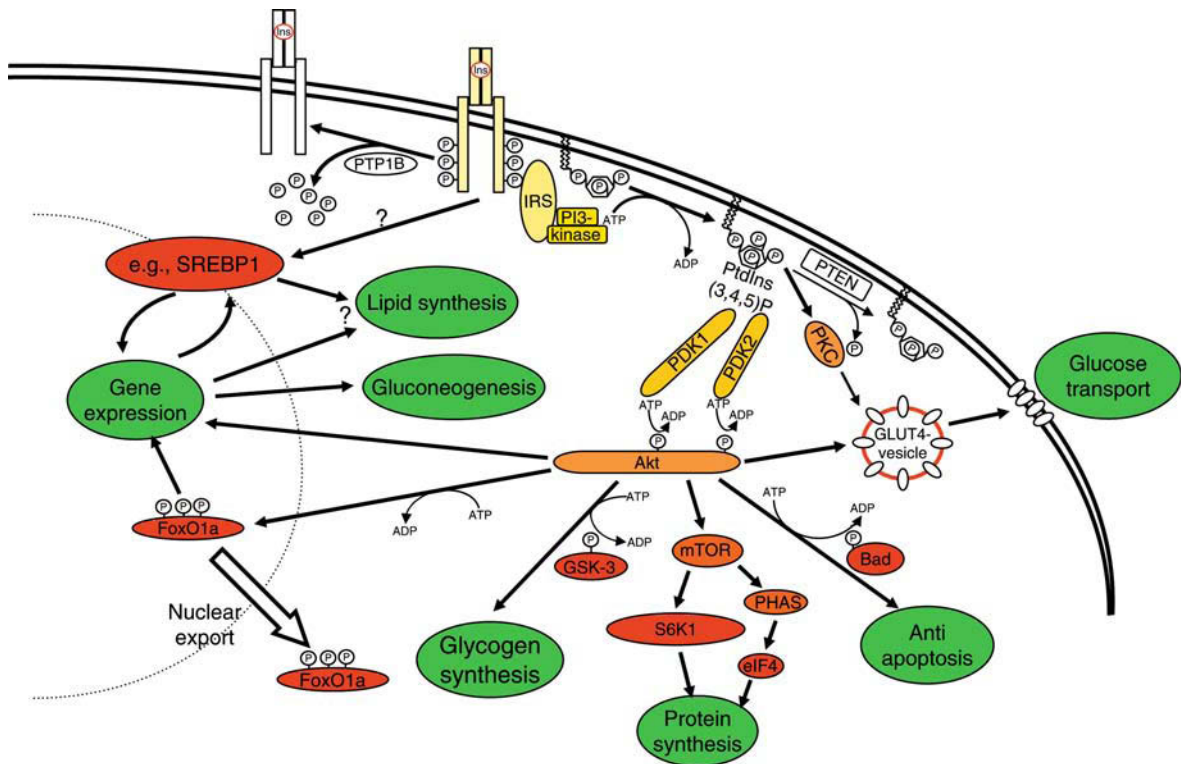
Organ	Effect	Mechanism	Comments
Liver	Activation of glycogen synthesis	Activation of glycogen synthase (GS)	Inactivation of glycogen synthase kinase 3 (GSK3) through phosphorylation by Akt
	Inhibition of gluconeogenesis/increased glycolysis	Inhibition of gluconeogenic gene expression/activation of some glycolytic enzymes	Akt-dependent inhibition of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) gene expression
			Decrease of cAMP, the second messenger of glucagon. Induction of pyruvate kinase and glyceraldehyde dehydrogenase
Fat	Increased glucose transport	GLUT4-translocation	PI 3-kinase/Akt mediated translocation of GLUT4 into the plasma membrane. Potential involvement of atypical forms of protein kinase C (PKC ζ and λ)
	Increased lipid synthesis/inhibition of lipolysis	Activation of lipoprotein lipase (LPL)/induction of fatty acid synthase (FAS)/inactivation of hormone sensitive lipase (HSL)	Facilitated uptake of fatty acids by LPL-dependent hydrolysis of triacylglycerol from circulating lipoproteins. Increased lipid synthesis through Akt-mediated FAS-expression. Inhibition of lipolysis by preventing cAMP-dependent activation of HSL (insulin-dependent activation of phosphodiesterases?)
Skeletal muscle	Increased glucose transport	GLUT4-translocation	see above (fat)
	Activation of glycogen synthesis	Activation of glycogen synthase	see above (liver)
	Increased protein synthesis	Increased amino acid uptake/increased translation of mRNA	Akt-mediated stimulation of system A amino acid transporter and stimulation of mRNA-translation through activation of p70S6kinase and elongation initiation factor 4 (eIF4). Possible involvement of atypical PKCs
Central nervous system	Reduction of body weight	Reduced appetite	changed expression of anorexigenic (e.g., POMC) and orexigenic (e.g., NPY) hypothalamic neuropeptides

Other, more general effects of insulin on cellular function include stimulation of cell growth (increase in DNA and protein synthesis), inhibition of apoptosis, and modulation of ion-channel activity.

adipocytes, β -cells, and hepatocytes; IRS4 mRNA is detected in thymus, brain, and kidney. Taken together, the data suggest that the major effects of insulin on metabolism are mediated via IRS1 and IRS2 [2, 3].

Tyrosine phosphorylated IRS interacts with and activates $\blacktriangleright$ PI 3-kinase [3]. Binding takes place via the SRC homology 2 (SH2) domain of the PI 3-kinase regulatory subunit. The resulting complex consisting of INSR, IRS, and PI 3-kinase facilitates interaction of the activated PI 3-kinase catalytic subunit with the phospholipid substrates in the plasma membrane. Generation of PI 3-phosphates in the plasma membrane recruits phospholipid dependent kinases (PDK1 and PDK2) which subsequently phosphorylate and activate the serine/threonine kinase $\blacktriangleright$ Akt (synonym: protein

kinase **B**, PKB). Three isoforms of Akt have been identified (Akt1, 2, 3). AKT1-knockout mice exhibit growth retardation and increased apoptosis, but no increased prevalence in diabetes. Disruption of the Akt2 gene produces diabetes due to insulin resistance of skeletal muscle and liver. Double knockout of AKT1 and 2 showed severe growth deficiency and died shortly after birth. There is solid evidence that activation of Akt is essential for the effect of insulin on glucose transport ($\blacktriangleright$ GLUT4), glycogen synthesis, $\blacktriangleright$ gluconeogenesis, protein synthesis, and gene expression [1, 3] (summarized in Table 1 and Fig. 2). Substrates of Akt are $\blacktriangleright$ glycogen synthase kinase 3 (GSK3) and $\blacktriangleright$ FoxO1a (previously known as FKHR), a transcription factor regulating the expression of gluconeogenic enzymes.



Insulin Receptor. Figure 2 Signal transduction of the insulin receptor. Activation of the insulin receptor leads to stimulation of the PI 3-kinase pathway. Generation of 3'-phosphorylated PI-phospholipids mediates the effect of insulin on glucose transport, cell survival, protein synthesis, glycogen- and lipid synthesis, gene expression, and gluconeogenesis. The protein kinase Akt plays a central role in the regulation of these PI 3-kinase dependent processes.

Also, phosphorylation of Akt results in activation of sterol regulatory-element binding protein 1 (SREBP1), a key transcription factor involved in regulation of lipogenic enzymes. In addition, some of the effects of insulin on cell proliferation and survival may be explained by an Akt-dependent inhibition of **apoptosis** through phosphorylation and inactivation of proapoptotic proteins (e.g., BAD, Caspase 9).

INSR signaling is terminated by specific phosphotyrosine phosphatases (e.g., PTP1B) (**tyrosine phosphatases**), and mice lacking the PTP1B gene exhibit increased insulin sensitivity. Furthermore, it has been suggested that the lipid phosphatases PTEN and SHIP2, the ligand-induced endocytosis and degradation of the activated INSR, and the degradation of insulin by insulin-degrading enzyme (insulysin) are involved in the termination of insulin signaling.

Drugs

Agents Stimulating the Receptor or the Signaling Pathway

Insulin Analogs. At present, the only known ligands of the insulin receptors are insulin isotypes from different species and a number of synthetic analogs

with insulin-like activity. Five analogs generated by site-directed DNA mutagenesis are used in clinical practice because of their pharmacokinetic characteristics: Insulin lispro (swap of proline B28 and lysine B29), insulin aspart (generated by exchange of proline B28 for aspartate), insulin glulisin (generated by exchange of lysine B29 for glutamate) are rapid and short-acting insulins (**diabetes mellitus**). Insulin glargine and insulin detemir are long-acting analogs. Because of two additional arginines at B31 and B32, insulin glargine is soluble at low pH and precipitates after injection, forming a stable, long-acting depot. Insulin detemir is a truncated insulin derivative (threonine B30) that has been coupled to a long-chain fatty acid at lysine B29. This results in the formation of a slowly dissociating complex and an increased binding to albumin, which further delays its inactivation.

Thiazolidinediones (synonyms glitazones, insulin sensitizers; rosiglitazone, pioglitazone) are a novel class of oral antidiabetic drugs that activate the transcription factor **peroxisome proliferator-activated receptor** (PPAR γ). Thiazolidinediones ameliorate insulin resistance in obese animal models and in individuals

with type-2 diabetes. They enhance the insulin dependent activation of the PI 3-kinase/Akt pathway, stimulate differentiation of preadipocytes, and redistribute intra-abdominal and hepatic triglycerides to subcutaneous adipose tissue stores. The marked reduction of hepatosteatosis is believed to be the primary reason for its antidiabetic effect. Furthermore, anti-inflammatory properties of thiazolidinediones have been suggested to contribute to their effect on insulin resistance in type-2 diabetes.

Concanavalin A is a plant lectin from the jack bean (*Canavalia ensiformis*) which binds with high affinity to mannose residues of glycoproteins. Concanavalin A is known to stimulate the tyrosine kinase activity of the INSR β -subunit with consecutive activation of kinases downstream the insulin receptor (IRS, PI 3-kinase). It is believed that Concanavalin A stimulates the activation and autophosphorylation of the INSR kinase through aggregation of the receptor, although the precise mechanism of action is unclear.

Vanadate (sodium orthovanadate or peroxovanadate) exhibits insulin-like effects in vitro (activation of insulin receptor tyrosine kinase, PI 3-kinase, Akt) and in vivo (diabetic rats, humans). These effects can be explained at least in part by the inhibition of phosphotyrosine phosphatases which deactivate the INSR tyrosine kinase.

Hydrogen peroxide (H_2O_2) exhibits insulin-like activity in isolated cells. Like that of vanadate, this effect is thought to be mediated by inhibition of protein-tyrosine phosphatases.

Agents Inhibiting Insulin Receptor Signaling

Insulin Analogs. Interestingly, several covalently dimerized insulin analogs are partial agonists of the insulin receptor. The intrinsic activity of the dimers decreases with the length of the spacer, with B29-B29'-suberoyl-insulin exhibiting the highest antagonist efficacy at the receptor [5].

Wortmannin is a fungus-derived inhibitor of PI 3-kinase. The agent binds and inhibits the enzyme covalently and irreversibly. It is very potent and considered to be highly specific (IC_{50} in most cells in the low nanomolar range).

LY294002 is a synthetic drug which reversibly inhibits PI 3-kinases. It is less toxic and also less potent than wortmannin. The IC_{50} in most cells is in the micromolar range.

Rapamycin is an immunosuppressive drug and an inhibitor of **►S6K1** (also known as p70S6-kinase) which phosphorylates ribosomal S6 protein. S6K1 is activated in response to insulin via activation of Akt. Rapamycin binds to a specific target protein (mTOR, mammalian target of rapamycin) which is functionally located downstream of Akt, but upstream

of S6K1. The IC_{50} in most cells is in the high nanomolar range.

- Tyrosin Kinases
- Phosphatidylinositol 3-Kinase
- MAP Kinase Cascades
- Peroxisome Proliferator Activated Receptor
- Diabetes Mellitus
- Diabetic Drop other than Insulin

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Insulin Resistance

Insulin resistance occurs when the normal response to a given amount of insulin is reduced. Resistance of liver to the effects of insulin results in inadequate suppression of hepatic glucose production; insulin resistance of skeletal muscle reduces the amount of glucose taken out of the circulation into skeletal muscle for storage; and insulin resistance of adipose tissue results in impaired suppression of lipolysis and increased levels of free fatty acids. Therefore, insulin resistance is associated with a cluster of metabolic abnormalities including: elevated blood glucose levels, abnormal blood lipid profile (dyslipidemia), hypertension, and increased expression of inflammatory markers (inflammation). Insulin resistance and this cluster of metabolic abnormalities is strongly associated with obesity, predominantly abdominal (visceral) obesity, and physical inactivity and increased risk for type 2 diabetes, cardiovascular and renal disease, as well as some forms of cancer. In addition to obesity, other situations in which insulin resistance occurs includes

pregnancy, infection or severe illness, stress, or during steroid use.

- Insulin Receptor
- Peroxisome Proliferator-Activated Receptors (PPARs)
- Diabetes Mellitus

Insulin Secretagogues

- Antidiabetic Drugs Other than Insulin

Insulin-like Growth Factor

Synonyms

IGF

Definition

IGF1 (also called somatomedin C) and IGF2 are ubiquitously expressed peptides with sequence homology to insulin. Both factors bind to specific receptors. The IGF1 receptor is a receptor tyrosine kinase with similar structure ($\alpha_2\beta_2$) and molecular function as the INSR. The receptor for IGF2 lacks a tyrosine kinase activity and is identical with the mannose-6-phosphate receptor. IGF1 also binds to the insulin receptor, although with much lower affinity; IGF2 binds to the IGF1 receptor with high affinity.

- Insulin Receptor

Integrase

Integrase is the enzyme of retroviruses that performs the incorporation of the viral DNA genome into the genome of the host cell. The enzyme is carried inside the viral particle and released into the host cell during infection. Upon back-transcription of the viral RNA genome into DNA by reverse transcriptase, integrase binds to the ends of this DNA product and removes two nucleotides. In a second step, integrase nicks the host cell genome and integrates the recessed viral nucleic acid into the cellular DNA.

- Viral Proteases

Integrin, α 4 β 1

The leukocyte integrin α 4 β 1 (also known as VLA-4 and CD49d/CD29) is a cell adhesion receptor, which is predominantly expressed on lymphocytes, monocytes and eosinophils. VLA-4 is generally selective for the CS1 domain within fibronectin, with an essential requirement for LDV sequence for binding. VLA-4 also binds to VCAM-1 as a counter receptor.

- Anti-integrins, Therapeutic and Diagnostic Implications

Integrin, α 4 β 7

The integrin α 4 β 7 is restricted to leukocytes and can bind not only to VCAM1 and fibronectin, but also to MAdCAM the mucosal addressin or homing receptor, which contains immunoglobulin-like domains related to VCAM-1.

- Anti-integrins, Therapeutic and Diagnostic Implications

Integrin, α IIb β 3

α IIb β 3 Integrin is a selective platelet integrin that generally binds to the RGD domain within fibrinogen and vWF. It mediates platelet-platelet aggregation that is essential for thrombosis and haemostasis.

- Anti-integrins, Therapeutic and Diagnostic Implications

Integrin, α v β 3

α v β 3 Integrin binds via RGD domain within various matrix proteins including vitronectin, osteopontin, and fibrinogen. It is widely distributed on various cells.

Integrin, α v β 5

α v β 5 Integrin generally binds to soluble and, with a higher affinity, to immobilized vitronectin via an RGD binding domain. α v β 5 integrin is expressed on endothelial, epithelial, and other cells.

► Anti-integrins, Therapeutic and Diagnostic Implications

Integrins

Integrins constitute a large family of α β heterodimeric cell surface, transmembrane proteins that interact with a large number of extracellular matrix components through a metal ion-dependent interaction. The term “integrin” reflects their function in integrating cell adhesion and migration with the cytoskeleton.

► Anti-integrins, Therapeutic and Diagnostic Implications
 ► Antiplatelet Drugs
 ► Inflammation
 ► Table appendix: Adhesion Molecules

Integron

A DNA sequence with conserved flanking regions of typically 59 nucleotides and the structural gene encoding the integrase, an enzyme capable of cutting and religating specific DNA sequences to accumulate resistance genes inside the integron. This physical linkage of different antibiotic resistance genes assures the coselection and simultaneous spread of multiple resistance determinants even in the absence of selective pressure.

► Bacterial Resistance to Drugs

Interactions

► Drug Interaction

Interfacial Inhibitors

Drugs that bind at the interface of two (or more) macromolecules. Drug binding takes place within an intermediate configuration of the macromolecular complex. The drug-stabilized macromolecular complex alters the function of the macromolecular complex and is recognized by the cell as a damaging intermediate. The paradigms for interfacial inhibitors are camptothecins for the topoisomerase I–DNA complex, which is trapped by camptothecins as topoisomerase I cleaves the DNA. A number of natural products act as interfacial inhibitors for a wide range of macromolecular complexes. They include brefeldin A, rapamycin, paclitaxel, vincristine, tubulin, and aminoglycosides (Pommier and Cherfil, Interfacial inhibition: a Nature’s paradigm for drug discovery. Trends Pharmacol Sci 2005; 28: 136–145).

► Camptothecins

Interferon Stimulated Gene 15

Synonyms

ISG15

Definition

ISG15 is one the genes most strongly induced by type I interferons. Transcription is also up regulated upon viral infection, oxidative stress and treatment with lipopoly-saccharide (LPS). Like ubiquitin, ISG15 can be conjugated to a wide range of target proteins. As mice lacking ISG15 are more sensitive upon herpes- and influenza-infections the ISG15 system apparently has a pivotal role in antiviral defense.

► Interferons
 ► Ubiquitin/Proteasome

Interferons

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 Germany

Synonyms

Antiviral immunoregulatory cytokines

Definition

Interferons (IFNs) are a family of multifunctional secreted proteins in vertebrates. Their most prominent functions are their antiviral properties on homologous cells against a wide range of viruses. It is important to note that prior exposure to IFN is required to render cells resistant to viral infection and replication. In contrast to antibodies, IFNs have no direct neutralizing effect on viruses.

In mammals the type I IFN genes form a large multigene family comprising the species α , β , κ , ω , and τ . The best-studied species is the IFN- α group, which comprises at least 14 closely related genes in man, and the single IFN- β gene. These species are rapidly induced by viruses or ►double-stranded (ds) RNA molecules. IFN- β is produced in almost all cell types, whereas the most efficient IFN- α -producing cells are ►plasmacytoid dendritic cells (pDC). IFN- κ has been identified in ►keratinocytes where it is expressed constitutively without external stimuli. IFN- κ is also inducible by virus and by IFN- γ in monocytes and in ►dendritic cells (DC). IFN- ω constitutes a minor species of type I IFN co-induced by viruses with α - and β -IFN in leukocyte cultures. IFN- τ , formerly called trophoblastin or ovine trophoblast protein, was isolated from sheep and bovine embryonic tissues. This IFN species is not virus-inducible but is released constitutively by ►trophoblast cells during the period immediately prior to implantation and therefore seems to play a key role in the establishment of maternal recognition and of pregnancy in ruminants. All type I IFN species except IFN- κ are encoded by intronless genes on the short arm of human chromosome 9 or mouse chromosome 4 and bind to a common cell-surface receptor consisting of two distinct chains present on virtually all nucleated cells.

In contrast to the large group of type I IFN genes, type II IFN comprises only IFN- γ . The gene contains three ►introns, displays no homology to type I IFN and is expressed exclusively by T-lymphocytes and natural killer (NK) cells, following stimulation with certain interleukins (IL-2, IL-7, IL-12, and IL-18), ►mitogens or the specific antigen of sensitized T-lymphocytes. IFN- γ binds to its specific receptor as a homodimer. All natural type I and II IFNs are glycosylated, but non-glycosylated recombinant IFNs show the same spectrum of biological activities (►interferon measurement).

Type III IFNs, also named IFN- λ 1, - λ 2, and - λ 3 or IL-28A, B, and IL-29, respectively also display IFN-like activities and are induced by most viruses that also give rise to type I IFN production. The genes are located on human chromosome 1 and contain several introns. IFN- λ binds to two receptor chains distinct from type I and type II IFNs named IFNLR-1 and IL-10R2 the latter being a common chain for IL-10 and IL-22. Signaling

involves the Jak/“Signal-Transducer-and-Activator-of-Transcription” (STAT) pathway as all other IFNs and most interferon-stimulated genes (ISG) are also induced by IFN- λ species.

This essay will focus on IFN- α , - β , and - γ .

Mechanism of Action

Regulation of IFN Gene Expression

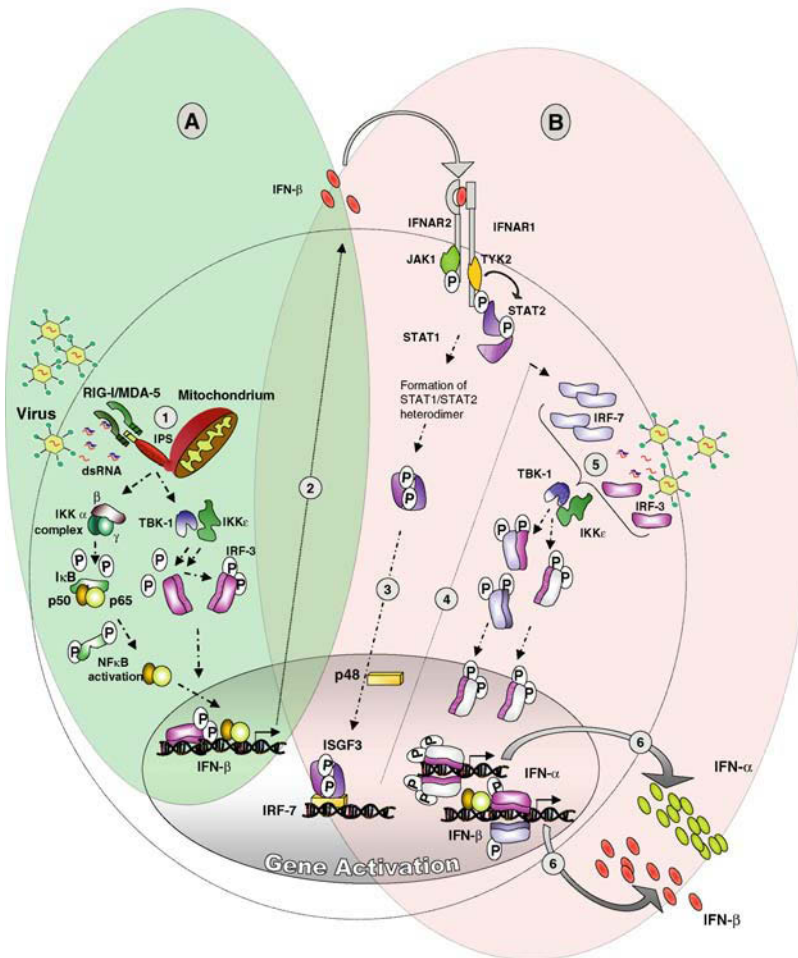
Type I IFN Induction

Type I IFNs are considered an integral part of the innate defense system (►innate immunity) against viral infection. Large amounts of IFN are produced in many cell types upon infection by various viruses, and production is tightly controlled at the transcriptional level. The predominant and best-studied species are IFN- α / β . Generally speaking, four major pathways can be considered as giving rise to activation of IFN- α and/or IFN- β and they are operating in a cell-type and virus-specific manner.

In the so-called “classical” pathway active in most cell-types, infection is detected as soon as viral replication is initiated and dsRNA intermediates are generated in the cytosol of the host cell. In addition to the dsRNA-dependent protein kinase (PKR), two RNA ►helicases, Retinoic-Acid-Inducible-Gene I (RIG-I) and Melanoma-Associated-Antigen 5 (MDA5), have been identified as the first cytoplasmic sensors of dsRNA. Structurally, RIG-I contains two and MDA-5 one ►caspase recruitment domains (CARD) enabling them to interact with the CARD of an adaptor molecule named Interferon-Promoter-Stimulator (IPS-1), MAVS, VISA, or Cardif anchored in the outer mitochondrial membrane [5]. This adaptor protein then recruits two different sets of kinases involved in downstream signaling: (i) the inhibitor of κ B (IKK)-related kinases TBK-1 and IKK ϵ which ultimately lead to phosphorylation of ►interferon-regulatory-factors (IRF) 3 and 7 and (ii) the I κ B-kinase complex consisting of IKK- α / β / γ subunits which phosphorylate the inhibitor I κ B, and mediate the release and activation of NF κ B (see also Fig. 1). Both sets of activated transcription factors translocate to the nucleus and bind to the IRF/PRDI and NF κ B/PRDII elements of the IFN- β promoter. These coordinated pathways then result in a moderate but rapid IFN- β response. The serine-threonine protein kinase PKR is also involved in virus-induced IFN synthesis since PKR-deficient cells show impaired IFN response to dsRNA and some RNA viruses. Pretreatment with IFN, however, completely relieves this defect, suggesting an involvement of the IFN-inducible helicase RIG-I in PKR-mediated IFN synthesis

An additional pathway leading to very efficient IFN- β responses involves one of the ►Toll-like-receptors (TLR), TLR3 which also senses dsRNA and is mainly expressed in ►dendritic cells (DC) and macrophages. Signal transduction then proceeds via the adaptor molecule TICAM/TRIF associated with the ►TIR-domain of

Virus infection results in a two-step induction of type I IFN



Interferons. Figure 1 The “Classical pathway”: virus infection results in two-step induction of type I IFN [4]. (A) *Green transparent*: (1) Following viral entry into the cell dsRNA intermediates formed during replication are recognized by RNA-helicases RIG-I or MDA-5. These helicases then interact with a signaling intermediate localized to the mitochondrial membrane called Interferon-Promoter-Stimulator-1 (IPS-1) via their common Caspase Activation and Recruitment Domains (CARD). This interaction initiates the formation of active kinase complexes containing IKK ϵ and TBK-1, which phosphorylate IRF-3 whereas those harboring IKK α , - β , and - γ mediate activation of NF κ B through phosphorylation and release of the bound inhibitor I κ B. NF- κ B and phosphorylated IRF-3 then translocate to the nucleus to stimulate the IFN- β promoter. This first step is a rapid process independent of protein synthesis due to the constitutive expression of IRF-3 with low to moderate yields of IFN- β . As can be seen from this simplified schematic view, IPS has a central role in initiating antiviral responses through both TLR3 and RIG-I-mediated pathways. (B) *Red transparent*: (2) In a positive feedback regulation the secreted “early” IFN- β then stimulates the type I IFN receptor and – via formation of interferon-stimulated-gene-factor 3 (ISGF3) – activates the IRF-7 gene through binding to its ISRE element (3). In concert with IRF-3 de novo produced IRF-7 (4) is also phosphorylated in the cytoplasm during ongoing viral infection (5), and following nuclear translocation as homo- or heterodimers both IRF-3 and IRF-7 activate IFN- α and IFN- β gene promoters. This second step is highly efficient resulting in an amplified “bulk” IFN response (6).

TLR3, which directly activates the TBK-1, not involving the CARD-containing adaptor IPS-1. Again the final step is activation of IRF-3.

The third pathway for IFN-induction involves detection of microbial nucleic acids by other types of

TLRs and is highly active in pDC which are robust producers of IFN- α and represent crucial effector cells of the innate defense system.

They express in endosomal compartments TLRs 7, 8, and 9 which specifically recognize ssRNA and

CpG-rich dsDNA, respectively. Following activation, these TLRs bind to a complex consisting of the specific adaptor molecule MyD88, TRAF6, and the kinases IRAK-1 and IRAK-4. IRF-7, which is also part of this complex is then phosphorylated by IRAK-1, forms dimers and shuttles to the nucleus to specifically activate the IFN- α promoter.

Efficient inducers of type I IFN also include bacteria or ►lipopolysaccharide (LPS) derived from gram-negative bacteria. LPS is mainly recognized by ►TLR4 on the cell membrane of monocytes and granulocytes. Signaling is then mediated via MyD88 for ►TNF- α and ►IL-6 or via TRIF/TICAM and IRF-3 for IFN- β responses. Interestingly, the gram-negative bacteria *Legionella pneumophila*, which are able to replicate in TLR4 negative human epithelial cells, induce IFN- β through activation of IPS-1 and IRF-3, a process that bypasses helicases RIG-I and MDA5.

Initiation of transcription requires activation of the IFN- α and - β ►gene promoters located 110–40 base pairs upstream of the start codon. The events leading to activation of the IFN- β promoter have been studied in detail. This promoter is composed of an overlapping set of four regulatory elements, two of which are target sequences for members of the IRF family of ►transcription factors. This family is comprised of nine members with remarkable homology in the DNA-binding domain, and IRF-3 and IRF-7 being crucial for inducing maximal type I IFN expression. The strong IFN- α/β response measured after viral infection is critical for host survival. As explained in Fig. 1, this response is due to a positive feedback regulation and is generated in a two-step process [4]. IRF-7 has been shown to represent the key element since the gene carries a sequence named “Interferon-Stimulated-Response-Element” (ISRE) – a conserved sequence in the regulatory region shared by most IFN-inducible genes – and also participates in the induction process through binding to the IFN- β promoter. Due to the pronounced effects IFNs have on the regulation of cell growth and immune functions, the duration of “bulk” synthesis is generally limited. Two mechanisms are known to mediate rapid post-induction turnoff of IFN- β gene expression: First, as a result of negative feedback mechanisms virus-inducible and even IFN-inducible competitive factors downregulate the IFN- β promoter activity as for instance, members of the SOCS-protein family and LGP2, another helicase protein lacking a CARD but able to interact with the IPS-1/RIG-I complex to displace IKK ϵ and inhibit IRF-3 activation (see above); second, IFN- β mRNA has a rapid turnover due to an AUUUA motif in the 3'-non-coding region, conserved in many different cytokine mRNA. In addition, in non-induced cells repressor proteins prevent transcriptional activation of the IFN- β promoter.

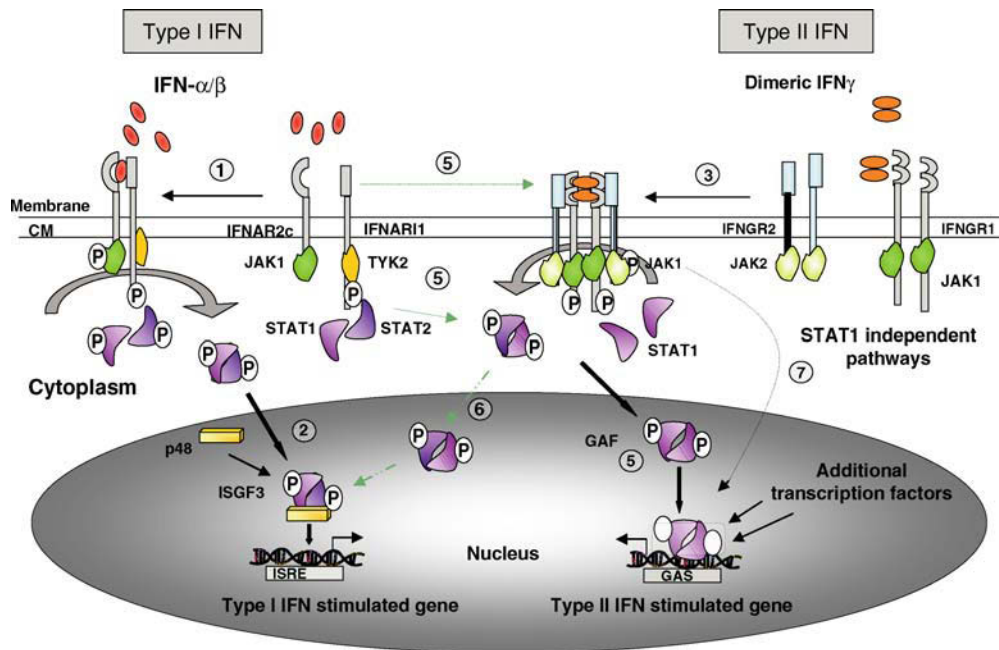
Type II IFN Induction

In contrast to IFN- α/β , the expression of IFN- γ is strictly limited to T-cells and large granular lymphocytes, also often referred to as ►natural killer (NK) cells. In naive T-lymphocytes, the capacity to express IFN- γ is gained during differentiation to the T_H1 lineage driven by specific antigen contact and requires the presence of unique transcription factors T-bet and Runx3 that bind to the *ifng* promoter. At the same time these factors repress the expression of IL-4 – a cytokine secreted by T_H2 lymphocytes and known to stimulate several immune cell functions antagonistic to IFN- γ – through binding to the *il4* silencer region. In addition, in cells that do not express IFN- γ a silencing effect by ►DNA methylation has been postulated, since a CpG target region in the IFN- γ promoter near the ►TATA box was found to be methylated (►immune defense). Hypomethylation per se, however, does not result in IFN- γ expression. It is therefore assumed that in unstimulated cells nuclear factors keep the gene in the “off” position since continuous production of this highly active inflammatory cytokine would be harmful to the host by eliciting autoimmune responses (►inflammation).

Signal Transduction and Biological Responses to IFNs

Any IFN-mediated activity requires binding to a specific receptor on the cell surface. The number of receptors varies from several hundreds to a few thousands per cell. All IFN- α species and IFN- β share the same type I IFN receptor to which they bind with similar affinity. IFN- γ binds to the distinct type II receptor. Both receptors are composed of one ligand-binding and one non-ligand-binding chain that associate, following contact with the specific IFN ligand. The signaling events following type I and type II IFN receptor stimulation are depicted on a simplified scheme in Fig. 2. Crucial components of this signaling pathway are the so-called ►signal transducer and activator of transcription (STAT) proteins, which are activated by phosphorylation on tyrosine residues.

Although IFN- γ specifically activates genes carrying a γ -activated-sequence (GAS) element in their regulatory region, it can also indirectly activate genes harboring an ISRE via binding of the transcription factor IRF-1, since IRF-1 is inducible by both IFN- α/β and IFN- γ . In addition, efficient IFN- γ signaling is dependent on simultaneous or prior stimulation of the IFN- α/β receptor that results from a cross-talk between IFNAR1 and IFNGR1 receptor components. This is borne out by observations that on one hand a low constitutive IFN- β expression observed in some cell types, such as macrophages and embryonic fibroblasts, considerably enhances their sensitivity toward



Interferons. Figure 2 Receptor stimulation and signal transduction by type I and type II IFN. *Type I IFN:* The receptor is composed of two chains, IFNAR1 and IFNAR2c their cytoplasmic domains carrying the tyrosine kinases TYK2 and JAK1, respectively, which are essential for signaling. In addition, unphosphorylated STAT2 is found attached to JAK1/IFNAR2c and stabilizes association of this receptor chain with STAT1. Upon IFN- α/β binding, the receptor complex is formed (1), and bound IFN is internalized via receptor-mediated endocytosis. The receptor associated kinases JAK1 and TYK2 get into closer contact and are activated by transphosphorylation. Subsequently, TYK2 phosphorylates a tyrosine residue of the IFNAR1 subunit, which in turn recruits and phosphorylates STAT2. STAT1 molecules will then be recruited to the phosphorylated STAT2 to become phosphorylated as well. The phosphorylated STAT1/STAT2 heterodimer thus formed finally dissociates from the IFNAR1 chain and translocates to the nucleus where it associates with the DNA-binding protein p48/IRF-9 (2) to form the trimeric complex ISGF3. The ISGF3 complex binds to a conserved target sequence in the promoter of IFN-inducible genes. This ISRE is essential for activation by IFN- α/β . Interestingly, the ISRE shares significant homology with defined domains in the type I IFN promoter and can therefore also be stimulated by members of the IRF family. *Type II IFN:* The type II (IFN- γ) receptor is composed of two subunits, which are associated with the kinases JAK1 and JAK2. After binding of the dimeric IFN- γ the chains oligomerize (3). In contrast to type I IFN signaling pathway, the IFNGR-associated kinases only recruit STAT1 molecules to a C-terminal IFNGR1 sequence containing several tyrosine residues phosphorylated previously. The STAT1 molecules are also phosphorylated and dissociate from the receptor to form a homodimer (4) known as γ -activated-factor (GAF), which in the nucleus recruits additional transcription factors to form the STAT1 coactivator complex and interacts with defined elements of IFN- γ -inducible gene promoters named γ -activated-sequences (GAS) to stimulate gene expression. In cells constitutively secreting IFN- α/β – such as macrophages and embryonic fibroblasts – the IFNAR1 chain is constitutively phosphorylated. Through association with the nonligand-binding chain IFNGR2 (5) the phosphorylated IFNAR1 contributes to type II IFN signaling by providing phosphorylated STAT2 molecules that enable ISGF3 assembly (6) and activation of the ISRE in type I IFN-inducible gene promoters. IFN- γ is also able to activate genes independently of STAT1. This signaling pathway is dependent on JAK1 and JAK2 (7).

IFN- γ , whereas on the other hand the IFN- γ induced antiviral activity is strongly reduced in cells lacking the IFNAR1 chain.

STAT signaling occurs within minutes following activation but also underlies rapid deactivation by cytoplasmic phosphatases or antagonistic factors such as members of the SOCS-proteins themselves induced by activated STATs. Another group of proteins called PIAS (proteins that inhibit active STATs) inhibit binding of activated STATs to their target DNA sequences

whereas members of the family of ubiquitin ligases promote proteasomal degradation of STAT molecules.

Oligonucleotide arrays ([▶microarray technology](#)) using cRNA prepared from IFN- α/β and IFN- γ treated cells have identified >300 inducible genes, displaying cell-type specific variations. Not all of these genes are exclusively inducible by IFNs, some of them being also activated by virus infection, dsRNA, LPS, or other cytokines. A number of genes, in particular those involved in cell proliferation, are inhibited by type I IFN.

The antiviral properties of IFNs against many viruses with different infection and replication strategies often rely on the concerted action of many induced genes and their molecular mechanism still remains obscure. In some instances, however, the antiviral activity against particular viruses can be attributed to specific genes. Depending on the type of virus, there is evidence for IFN-mediated inhibition of virus uptake by the cell, inhibition of viral transcription, of viral protein synthesis, or of virus maturation, and release from the cell. More specifically, IFN-induced genes include the IRF family of transcription factors, a wide range of enzymes, e.g., the 2',5'-oligoadenylate-synthetase (2',5' OASE), RNaseL, and the protein kinase PKR (see below) as well as factors governing immune responses. Among these are the ►proteasome subunits LMP2 and LMP7 and ►MHC molecules that are essential for antigen processing and presentation, respectively. Type I IFNs also non-specifically induce the proliferation of CD8⁺ memory T-cells that interact with antigen-presenting cells to elicit immune responses. This effect is indirect, mediated through the secondary induction of IL-15. Interestingly, type I IFNs also exert growth arrest and thus prevent the induction of apoptosis in both CD4⁺ and CD8⁺ memory T-cells. This fine-tuned balance seems to depend on the amount of IFN secreted from fibroblasts, stromal cells, and monocytic cells and contributes to the long-lasting persistence of memory T-cells in the immune system. Dysregulation of its synthesis, however, may prevent elimination of autoreactive T- and B-cells and may be associated, for instance with T-cell-dependent chronic joint inflammation or the presence of autoreactive antibodies.

IFN-induced proteins involved in cell growth inhibition are considered ►tumor-suppressor genes [1] as for instance p53 and its downstream target gene, the cyclin-dependent kinase inhibitor p21/waf, the transcription factors IRF-1, IRF-8, and members of the p200 family. The latter inhibit several transcriptional activators including the ►proto-oncogenes c-myc, c-fos, and c-jun and bind to the retinoblastoma protein (Rb), which is a major player in cell-cycle regulation (►Cell Cycle Control). Among the IRF group of transcription factors, "Interferon-Consensus-Sequence-Binding-Protein" (ICSBP/IRF-8) is specifically induced by IFN- γ and its expression is limited to cells of monocytic and lymphoid origin. Cytogenetic analyses have provided evidence for tumor suppressor activities of both IRF-1 and ICSBP/IRF-8. Blood samples from patients with chronic myelogenous ►leukemia (CML) exhibited impaired ICSBP expression, whereas deletion of the *ICSBP* gene in mice led to a CML-like syndrome. Chromosomal deletions or point mutations within the IRF-1 locus are among the most frequent cytogenetic abnormalities in myeloid leukemia and myelodysplastic syndromes,

and experimental deletion of the IRF-1 gene facilitates oncogenic transformation in vitro. The tumor suppressor gene p53 is induced by IFN and once activated by multiple phosphorylation – an IFN-independent process, mainly mediated through exogenous cellular stress factors – acts as a transcriptional regulator [3]. A salient feature of activated p53 is enhanced ►apoptosis of transformed cells.

From a certain size, tumor growth is dependent on blood vessel supply, a process defined as ►angiogenesis. This process is dependent on growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) and involves dissociation of endothelial cells and basement membranes of pre-existing vessels by way of matrix ►metalloproteinases (MMPs). IFNs are known to mediate angiostatic effects by inhibition of bFGF and MMP expression in tumor cells and induction of the chemokine CXCL10 (IP-10) in macrophages and may thus contribute to inhibition of tumor spreading.

A complicated interplay exists between both types of IFN and ►nitric oxide (NO), the details of which are still not clear. NO generated by ►inducible nitric oxide synthase (iNOS) in macrophages in response to IFN- γ plays an important role in host defense against viruses, bacteria, and eukaryotic parasites but has also been implicated in the pathogenesis of chronic inflammatory syndromes. In contrast as revealed by in vitro studies, type I IFN displays regulatory functions on iNOS induction elicited by pathogens: enhanced expression in the presence of both pathogen and IFN but inhibition following pretreatment with type I IFN.

IFN-induced mediators of antiviral activity include a large number of proteins. The best studied are the Mx proteins, the kinase PKR, and guanylate-binding proteins (GBP) displaying GTPase activity. Additional IFN-induced genes with potent antiviral activity are ISG20, coding for a cytosolic RNase specific for several ssRNA viruses and ISG15, coding for a secreted ubiquitin-like protein.

The pathway leading to 2',5' OASE/RNaseL activation also contributes to the antiviral and antiproliferative effects of IFN- α/β . Both are induced by IFNs but require dsRNA or viral infection for activation. 2',5' OASE polymerizes ATP into 2',5'-linked oligoadenylates (2–5A) of different lengths. In turn, these molecules bind to and activate the latent RNaseL, which is also involved in IFN-mediated apoptosis. Much attention has also been attributed to the serine-threonine kinase PKR. In the presence of dsRNA, PKR undergoes autophosphorylation and phosphorylates selected cellular proteins such as the translation-initiation factor eIF2 α . Phosphorylation inactivates eIF2 α leading to an arrest of cellular protein synthesis. Most importantly, PKR displays the properties of a tumor suppressor since its inactivation in vitro causes malignant transformation

and prevents apoptosis of virus-infected cells. In addition to PKR and RNaseL, a variety of other proteins participate in the initiation of ►apoptosis; for instance p53, IRF-1, and TNF-Related-Apoptosis-Inducing-Ligand (TRAIL) induced by type I and type II IFN. The selective induction of TRAIL in T-cells and fibroblasts may also represent an important component of host defense against virus-infected or tumor cells. Due to its rather specific action on malignant cells and few adverse effects *in vivo*, TRAIL is a promising anti-tumor agent for clinical trials. Finally, in a tumor line a heterogenous group of IFN- γ induced Death-Associated-Proteins (DAP) has been identified and characterized. Each of these findings reveals that programmed cell death is part of the IFN-mediated cellular defense and reveals that elimination of infected cells is an efficient means for an organism to combat virus infection.

Type I and type II IFNs are also involved in bone homeostasis by regulating the differentiation of ►osteoclast progenitors. In these cells differentiation is mediated by the “receptor activator of NF κ B ligand” (RANKL), a cytokine belonging to the ►tumor necrosis factor (TNF) family. IFN- γ inhibits one of the downstream targets of RANKL, TRAF6 (TNF-receptor activated factor) thus preventing activation of NF κ B and c-jun kinase JNK. Most interestingly, another RANKL-signaling factor is the protooncogene c-fos which not only promotes osteoclastogenesis but also stimulates IFN- β expression via a novel mechanism probably involving the AP-1 transcription factor complex. In a negative feedback mechanism, IFN- β suppresses c-fos expression which ultimately maintains a fine-tuned balance of bone metabolism. Accordingly, IFN- β or *IFNARI*-deficient mice show disturbed trabecular bone architecture with reduced bone mass.

Clinical Use

IFNs were the first therapeutic products resulting from recombinant DNA technology. In view of their pronounced antiproliferative effects, crude preparations of natural leukocyte IFNs were already being used for the treatment of various tumors well before the availability of recombinant material. However, due to limited supply, the number of patients and duration of treatment did not allow for the evaluation of long-lasting beneficial effects. Today, numerous clinical trials have been conducted for a variety of different human diseases. Based on the results, approved indications have been formulated for the therapeutical application of IFNs (Table 1). Most of the responding tumors are of hematopoietic origin. IFN- α has therapeutic potentials for multiple myeloma, low-grade non-Hodgkin lymphoma, cutaneous T-cell lymphoma, and adult T-cell lymphoma. The overall response rates in hematological tumors were comparable to ►chemotherapy. Combination regimen mainly using recombinant IFN- α 2 and chemotherapeutic drugs yielded promising results with even higher rates of complete hematologic remissions and survival. For patients with hairy cell leukemia, a rare chronic lymphoproliferative disorder, IFN- α is the therapeutic agent of choice with overall response rates of 75%. Generally used treatment schedules consist of daily parenteral application for 6 months followed by maintenance treatment 3 times weekly for 1 year. Fortunately, in patients with hematologic relapses, readministration of IFN- α is successful in most cases. Good response rates have also been obtained for IFN- α treatment of CML. This hematopoietic stem cell malignancy is characterized by a reciprocal translocation of chromosomes 9 and 22, giving rise to the constitutive expression of the chimeric

Interferons. Table 1 Approved indications for interferon therapy either as adjuvant or for monotherapy

Malignancy class	IFN- α	IFN- β
Hematological malignancies	Hairy cell leukemia	
	Chronic myelogenous leukemia	
	Multiple myeloma	
	Low-grade non-Hodgkin-lymphoma	
	Adult T-cell lymphoma	
	Cutaneous T-cell lymphoma	
Other malignancies	Metastatic renal cell carcinoma	
	Metastatic melanoma	
	Kaposi sarcoma	
Virus-associated benign tumors		Anogenital warts
		Juvenile laryngeal papilloma
Viral diseases	Hepatitis B	
	Hepatitis C	
Diseases of unclear etiology		Multiple sclerosis

BCR-ABL tyrosine kinase that is essential for malignant transformation. Cytogenetic responses to IFN- α therapy were seen in 30–40% of the treated patients with complete responses in about 10%. Long term survival can therefore be expected in these patients. In 2000, the BCR-ABL tyrosine kinase inhibitor Imatinib has been introduced for CML therapy and meanwhile has proven more efficient than IFN- α therapy.

Metastatic renal cell carcinoma has a poor prognosis and resists conventional chemotherapy. Immunotherapy with IL-2 and/or IFN- α is currently regarded as the most effective therapy with, however, modest response rates of 15–20%. Similar results are also observed in patients with metastatic melanoma and the response to IFN- α and IL-2 correlates with the occurrence of tumor-infiltrating CD4⁺ T-lymphocytes identified in aspirates from melanoma metastases. Determination of these cells therefore seems to be a method to predict responders prior to the initiation of cytokine therapy.

Kaposi sarcoma (KS) – an angiogenic-inflammatory neoplasm – is the most prevalent cancer in ►HIV-infected patients and its appearance is preceded by infection with human Herpesvirus-8 (HHV-8). Although chemotherapy has become the treatment of choice approved by the FDA, there are also good response rates in patients treated with IFN- α . Fortunately, today highly active anti-retroviral therapy (HAART) has dramatically decreased the incidence of KS in AIDS patients.

Although there has been substantial success using IFN for the treatment of some cancers, until this point, the great majority of tumors are resistant or show an initial moderate response soon followed by disease progression under treatment. One likely reason for resistance is the progredient loss of susceptibility to IFN, which may be caused by downregulation of IFN receptors or perturbation of intracellular IFN-signaling pathways, a phenomenon also known from *in vitro* studies.

Since their discovery in 1957, type I IFNs have been noted to have protective effects against human viral infections. This is why, apart from malignancies, diseases of viral etiology have been successfully treated with IFNs. For anogenital warts, a commonly acquired sexually transmitted disease caused by Human Papilloma Viruses (HPV), the most effective response is obtained after intralesional administration of IFNs in contrast to systemic treatment that only prevents the development of new warts. Juvenile laryngeal papilloma is a rare but severe benign lesion also caused by HPV infection and mainly observed in childhood. Recurrence is a characteristic feature of this disease and calls for repeated surgical removal. Treatment with type I IFN has proven highly effective most importantly in preventing recurrence of lesions.

Successful treatment with IFN- α also includes patients with chronic ►hepatitis B virus (HBV) infections. Despite the availability of an efficient vaccine, chronic

HBV infection remains a major worldwide public health problem. The World Health Organization estimates that there are still 350 million chronic carriers of the virus, who are at risk of developing chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. The success of IFN- α treatment – mainly performed as combined treatment with adenine-arabinoside – has been measured by the normalization of liver enzymes, loss of HBe antigen and of detectable viral DNA in the serum of patients. It has been estimated from several clinical trials that as many as 40% of treated HBV patients would respond to therapy with IFN- α or combined treatment with nucleoside analogues and IFN- α .

Similar to HBV, infections with ►hepatitis C virus (HCV) have a high rate of progression from an acute to a chronic state that frequently leads to cirrhosis or hepatocellular carcinoma [2]. Monotherapy for HCV infection with IFN- α or combined therapy with ribavirin and IFN- α is associated with initial rates of response as high as 40%. The rates of sustained responses are, however, lower and also depend on the viral genotype. In patients infected with HCV genotype 2 or 3, the response was maximal after 24 weeks of treatment, whereas patients infected with genotype 1 – the most frequent in the USA and Europe – required a minimum treatment course of 48 weeks for an optimal outcome.

Some improvement of clinical symptoms has been obtained with IFN- β monotherapy of multiple sclerosis (MS) although a definite cure has not been reported, even after treatment for years at highest tolerable doses 3 times weekly. The beneficial effects of IFN- β consist of significantly reduced clinical attack and relapse rates and delay of disability progression and are clearly dose-dependent with highest doses achieving maximal clinical responses. This chronic inflammatory disease of the central nervous system (CNS) is assumed to result from autoaggressive T-cell-mediated immune responses to self antigens such as myelin basic protein (MBP) but also to neurons. Stimulated by proinflammatory cytokines like IL-1, TNF- α , IFN- γ , and cellular adhesion molecules these T-cells and also macrophages cross the ►blood–brain barrier and gain access to the CNS. The trans-endothelial migration process involves the release of MMP, which cleave type IV collagen, a component of the blood–brain barrier. ►Astrocytes and microglia in the CNS are activated by the infiltrating immune cells, resulting in a chronic inflammatory process with irreversible axonal damage. The mechanism of the favorable response of MS to IFN- β treatment most likely resides within its immunoregulatory effects on the autoreactive T-cells. In this context, the therapeutic effects most likely are repression of IL-2 receptor expression and of IL-2 dependent release of MMP-2 and MMP-9 in a dose-dependent manner, resulting in inhibition of T-cell migration across the blood–brain barrier. In addition,

IFN- β reduces the induction by inflammatory cytokines of ►adhesion molecules and of ►MHC class I and II complex on endothelial cells, a process preceding attachment and transendothelial migration of T-cells. These anti-inflammatory effects of IFN- β exemplify antagonistic actions of type I and type II IFN. There is, indeed, much clinical evidence for the involvement of IFN- γ in inflammatory processes – through activation of iNOS and subsequent secretion of NO – leading to the establishment of ►autoimmune diseases as for instance in rheumatoid arthritis.

On the other hand, IFN- α may also be involved in the activation of autoreactive T-cells as has been proposed for type I diabetes. An IFN- α inducible ►superantigen, encoded by the truncated envelope gene of a human endogenous retrovirus and specifically activating V β 7 T-cells, has been detected in pancreatic lesions from type I diabetes patients, infiltrated by V β 7 T-cells. Since IFN- α expression could be detected in pancreatic β cells in concert with persistent viral infections, there is a clear link between viral infections and autoimmunity via IFN- α -stimulated ►superantigen expression.

Side Effects of IFN Therapy

During clinical trials aimed at defining the optimal IFN dose for therapy, various side effects became manifest with increasing doses. The most common side effects that develop independently of the IFN preparation used are transient nausea, skin reactions, chills, and fever, the latter two often being managed by prophylaxis with ibuprofen and paracetamol. In addition, however, therapeutic effectiveness is compromised by more severe secondary effects such as myelosuppression, cardiomyopathy, and impaired renal function requiring limitation of IFN therapy. Thyroid dysfunction resulting in ►hypothyroidism is also a known complication during IFN therapy in 10–20% of patients and may be caused by an autoimmune reaction. Fatigue is a common symptom of hypothyroidism and accounts for the most frequent side effect, reported in up to 50% of treated patients. IFN-mediated fatigue is recognized as a complex neurophysiologic phenomenon that is not transient but worsens with ongoing IFN therapy and may finally result in cognitive slowing, general lethargy, and depression. Finally, a substantial number of patients develop neutralizing antibodies during IFN treatment and their presence is associated with a poor therapeutic response. Most of these antibodies are specific for nonglycosylated, recombinant type I IFN.

In addition, clinical observations in IFN- α -treated hepatitis C or melanoma patients have suggested a promoting effect of type I IFN in autoreactive skin disorders such as *Lichen planus*. This inflammatory skin disease was, however, not restricted to IFN- α treated patients but was found to be associated with expression by so far unknown mechanisms of the IFN-induced MxA

protein and the appearance in skin biopsies of both ►chemokine receptor CXCR3⁺ lymphocytes and cells expressing the CXCR3 ligand CXCL10/IP10, which also represents an IFN-induced gene.

Second Generation IFNs

Improvements in therapy are expected through the development of “second generation” IFN. Reduced immunogenicity and enhanced stability have been reported for pegylated IFN- α , a conjugate of recombinant IFN- α and monomethyl-polyethylenglycol. Pharmacokinetic studies comparing pegylated and nonpegylated IFN- α preparations have revealed an increase of biological half life from 4 to 30 h and a sevenfold delay in the mean elimination time. Currently, pegylated IFN- α 2 in combination with ►ribavirin is considered the treatment of choice for chronic hepatitis C. For IFN- β , a complex with the soluble portion of the ligand-binding receptor chain IFNAR2 was tested in preclinical studies and showed a prolonged clearance and potentiated biological effects.

- Cytokines
- JAK-STAT Pathway
- Immune Defense
- Antiviral Drugs

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Interleukin-1 (IL-1)

The interleukin-1 (IL-1) family of proteins currently comprises IL-1 α , IL-1 β , and the IL-1 receptor antagonist (IL-1RA). The biological activities of IL-1 are shared by IL-1 α and IL-1 β , whereas IL-1RA is a true receptor antagonist. IL-1 is a key player in acute and chronic inflammatory diseases. Whether IL-1 has a role in normal physiology is still unresolved. IL-1 can

activate a wide range of cells important in both immunity and inflammation and is a promising novel target of anti-inflammatory therapy.

- Cytokines
- Inflammation

Interleukin-2 (IL-2)

Interleukin 2 (IL-2) is a 15.5 kDa glycosylated protein produced by helper T-cells in response to an antigen and interleukin-1. Ligation of the IL-2 receptor (expressed by a number of lymphocytes) by IL-2 stimulates growth, differentiation and survival.

- Cytokines
- Immune Defense
- TOR Signalling

Interleukin-4 (IL-4)

A cytokine, secreted by TH2-cells, stimulates B-cells in different stages of their development. It may act as a growth factor or as a differentiation factor, causing B-lymphocytes to switch antibody to IgE. In T-cells it causes differentiation into TH2-cells.

- Cytokines
- Immune Defense
- Bronchial Asthma

Interleukin-5 (IL-5)

A cytokine, secreted by TH2-cells and mast cells, stimulates B-cell growth, acts as hematopoietic factor for growth factor eosinophils, and extends the life span of eosinophils.

- Cytokines
- Immune Defense
- Bronchial Asthma

Interleukins

- Cytokines

Intermediate Filaments

Intermediate filaments are present in most animal cells. They are composed of more than 50 proteins which are expressed in a cell-type specific manner. Their diameter is about 10 nm and thus between those of the larger microtubules and the smaller F-actin. They form scaffolds and networks in the cyto- and nucleoplasm.

- Cytoskeleton

Intermediate-density Lipoprotein (IDL)

Lipoprotein formed by hydrolysis of triglycerides in VLDL; elevated in type III hyperlipoproteinemia.

- Lipoprotein Metabolism

Intermittent Claudication

Cramping sensation in the leg or buttock precipitated reproducibly by walking or exercise that occurs as a result of decreased oxygen supply due to severe atherosclerotic disease of the peripheral vascular system. It typically subsides after a brief rest.

- Atherosclerosis

Internalization

Internalization is an agonist-induced endocytosis of membranous receptors which occurs in seconds to minutes. It involves the formation of receptor containing

vesicles (e.g., clathrin-coated pits) and is followed by an endosomal acidification permitting dephosphorylation of the receptor and dissociation of the agonist. Internalized receptors are recycled to the cell surface or degraded in lysosomes.

► Tolerance and Desensitization

International Normalized Ratio (INR)

The international normalized ratio (INR) is a method to standardize reporting of the ►prothrombin time, using the formula, $INR = (PT_{\text{patient}}/PT_{\text{control}})^{ISI}$, where PT indicates the prothrombin times (for the patient and the laboratory control), and ISI indicates the “international sensitivity index,” a value that varies, depending upon the thromboplastin reagent and laboratory instrument used to initiate and detect clot formation, respectively.

► Anticoagulants

Intima

The innermost layer of an artery, which consists of loose connective tissue covered by a monolayer of ►endothelium that resides on a basement membrane. In human arteries, the intima often contains resident smooth muscle cells even early in life. Atherosclerotic plaques form in the intima.

► Atherosclerosis

Intracellular Transport

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Definition

The term intracellular transport comprises both the correct targeting and the mechanism of transport of

newly synthesized proteins and lipids to their destination and their retrieval from organelles and the plasma membrane to maintain the structural and functional organization of a eukaryotic cell.

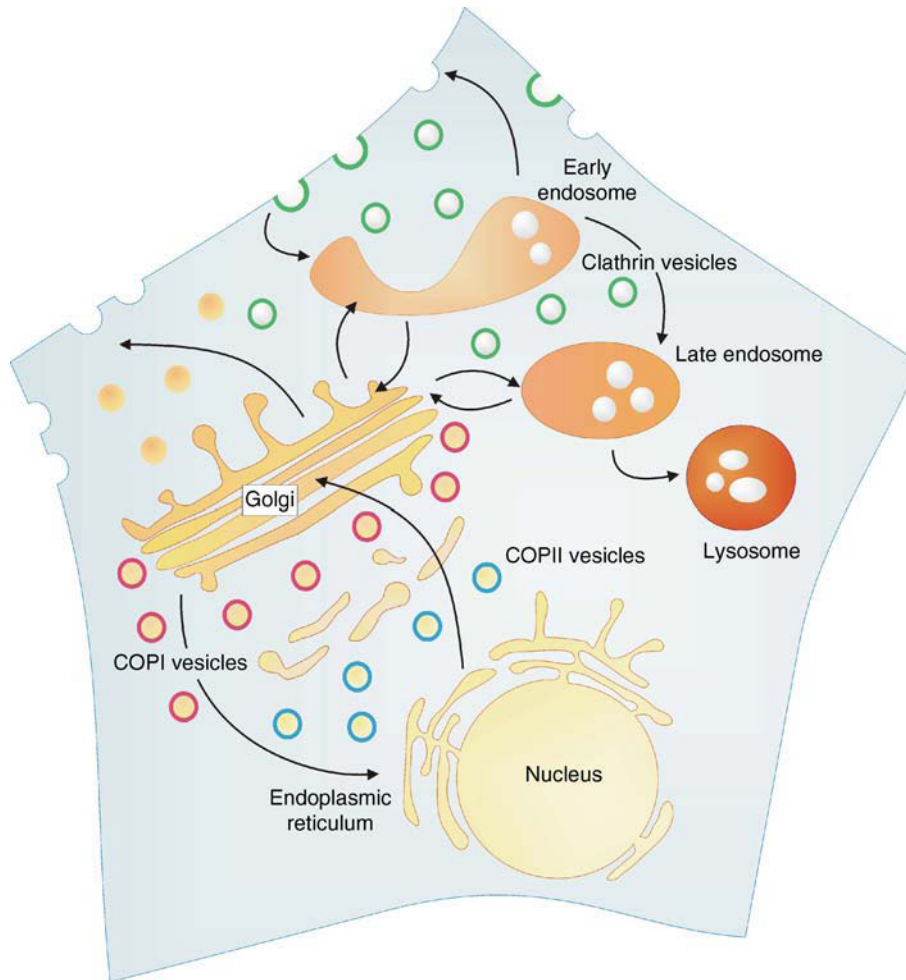
Basic Mechanisms

At any given time, a typical eukaryotic cell carries out a multitude of different chemical reactions, many of them mutually incompatible. Therefore, cells have developed strategies for segregating and organizing their chemical reactions. Thus, e.g., multi-enzyme complexes have evolved. Of particular importance in this respect are the membrane-bounded compartments, such as the nucleus, endoplasmic reticulum (ER), Golgi apparatus, lysosomes, endosomes, peroxisomes and mitochondria. The specialized functions of each organelle require distinct protein and lipid compositions. Since, a cell cannot usually make these organelles from de novo, information is required in the organelle itself. Thus, most of the organelles are formed from pre-existing ones, which are divided during the cell-division cycle and distributed between the two daughter cells. Thereafter, organelle growth needs a supply of new lipids and proteins. Even in resting cells, lipids and proteins must be continuously delivered to organelles; some for eventual secretion from the cell, and some to replace molecules that have been degraded due to their physiological turnover. Therefore, the problem of how to make and maintain organelles is largely one of how to direct newly synthesized lipids and proteins to their correct destinations.

The synthesis of virtually all proteins in a cell begins on ribosomes in the cytosol (except a few mitochondrial, and in the case of plants, a few chloroplast proteins that are synthesized on ribosomes inside these organelles). The fate of a protein molecule depends on its amino acid sequence, which can contain sorting signals that direct it to its corresponding organelle. Whereas proteins of mitochondria, peroxisomes, chloroplasts and of the interior of the nucleus are delivered directly from the cytosol, all other organelles receive their set of proteins indirectly via the ER. These proteins enter the so-called ►secretory pathway (Fig. 1).

Stations of The Secretory Pathway

The journey along the secretory pathway begins with the targeting of the nascent polypeptide chain to the ER by a hydrophobic signal sequence. This signal sequence interacts with the signal recognition particle complex and the growing polypeptide chain is translocated across the ER membrane to the ER lumen or inserted into the ER membrane via a translocation pore. On the luminal side of the ER, various chaperones associate with the polypeptide chain in order to mediate translocation and to control and support correct folding. In addition, the newly synthesized protein undergoes co- and post-translational modifications, i.e. glycosylation, disulfide



Intracellular Transport. Figure 1 Model of intracellular transport within a mammalian cell. Export from the ER to the IC occurs in COPII vesicles. Anterograde transport from the *cis*- to the *trans*-side of the Golgi is mediated by COPI vesicles. COPI vesicles are also involved in retrograde traffic from the Golgi to the ER. Sorting and transport at the exit side of the Golgi to organelles of the endocytic pathway and to the plasma membrane is mediated by clathrin vesicles. Additionally, other types of vesicles may be involved in sorting at the Golgi-exit.

bond formation and oligomerization. Once the protein is properly matured, it passes the quality control of the ER and exits the compartment via transport ►vesicles. Protein transport from the ER to the Golgi complex involves the ER-Golgi intermediate compartment (IC), also termed 15°C compartment or vesicular tubular clusters. Although the nature as a stable compartment of the IC is discussed controversially, it is generally accepted that the IC plays a role as a sorting station of anterograde and retrograde membrane flow. Subsequently, en route through the secretory pathway, the secretory proteins next enter the Golgi apparatus at the *cis*-Golgi network (CGN). They continue their passage through the several sub-compartments of the Golgi (CGN, *cis*-, *medial*-, *trans*-Golgi and *trans*-Golgi network (TGN)), where proteins are subjected to various kinds of post-translational processing, e.g. remodeling

of *N*- and *O*-linked oligosaccharide side chains, sialylation and tyrosine sulfation. Once a protein has reached the exit side of the Golgi, the *trans*-Golgi network, it has to be sorted to its final destination.

Transport Through The Secretory Pathway

The various transport steps between the stations of the secretory pathway are linked by a membrane flow, which is mediated by transport vesicles. In general, vesicles are formed from their donor membrane upon recruitment of coat proteins. Thereafter, the vesicles move to their target membranes where they dock. After uncoating they fuse with the target membranes. Export from the ER is the first step in the vectorial movement of cargo through the secretory pathway. The generation of vesicles from the ER is driven by the recruitment of a set of soluble proteins from the cytoplasm to the ER membrane that form a coat

structure, termed COPII. Components of the COPII coat have initially been identified as Sec (Sec for secretion) mutants of the yeast *S. cerevisiae* causing defects at distinct steps of intracellular transport. This led to the identification of three soluble protein components that make up the coat of ER derived transport vesicles: Sar1p, Sec23p complex and Sec13p complex. Sar1p is a small GTP-binding protein with a molecular mass of 21 kDa that shares primary structure identity with GTPases of the Ras family. The Sec23p complex is composed of two proteins: Sec23p, a 85 kDa protein and a tightly associated protein of 105 kDa called Sec24p. The Sec13p complex contains the 34-kDa subunit Sec13p and the 150-kDa protein Sec31p. The initial step of ►COPII vesicle formation is the recruitment of Sar1p-GTP to the ER membrane through a guanine nucleotide exchange reaction catalysed by the integral ER-membrane protein Sec12p. Next, the Sec23p complex is recruited, a prerequisite for binding of the Sec13p complex. As mentioned above, fusion of vesicles requires prior dissociation of the coat. Uncoating of COPII vesicles is achieved by hydrolysis of Sar1p-bound GTP, an action catalysed by Sec23p, part of the coat complex. GTP hydrolysis results in the dissociation of Sar1p from the vesicle membrane followed by the removal of the coat.

Anterograde transport of material from the *cis*- to the *trans*-side of the Golgi and also retrograde transport back from the Golgi to the ER is mediated via COPI coated vesicles. *In vitro* generation of ►COPI vesicles from isolated Golgi membranes has allowed their biochemical characterization. Components that make up the coat of COPI vesicles are the cytosolic proteins ADP-ribosylation factor 1 (ARF1) and ►coatamer, a stable heteroheptameric protein complex. ARF1, like Sar1p, belongs to the family of Ras GTP-binding proteins. The coatamer complex consists of seven different subunits termed [α , β -, β' -, γ -, δ -, ϵ - and ζ -COP (COP for coat protein)], present in a one by one stoichiometry in both the cytosolic and the membrane-associated form of the coatamer complex. Recently, an additional isoform of γ - and of ζ -COP was identified. The new isoforms are termed $\gamma 2$ - and $\zeta 2$ -COP, and the original isoforms are referred to as $\gamma 1$ - and $\zeta 1$ -COP. Each isoform is, like the other coatamer subunits, present in just one copy resulting in three different main heptameric protein complexes with only minor amounts of $\gamma 2 \zeta 2$ -coatamer.

Similar to COPII, the formation of COPI-coated vesicles is initiated by recruitment from the cytoplasm to the membrane of the GTP-binding protein ARF1. Membrane binding and activation of ARF1 is triggered by the exchange of GDP for GTP, a reaction that requires catalysis by a nucleotide exchange factor. Several exchange factors for ARF1 have been characterized to date, all of them are soluble proteins found in the cytoplasm. An important difference between COPI

and COPII is the GTP hydrolysis reaction needed for uncoating. While for COPII disassembly Sar1p-mediated GTP hydrolysis is activated by the coat protein Sec23p, acceleration of GTP hydrolysis in ARF1 needs the activation by ARF-specific GAPs (GTPase activating proteins), which are recruited from the cytoplasm to the membranes.

Along their route through the Golgi, secretory and membrane proteins destined for the various post-Golgi pathways are intermixed. Thus, proteins of distinct routes, i.e. the endosomal and the secretory route, are sorted into individual types of transport vesicles at the TGN. Among the best characterized types of TGN-derived vesicles are ►clathrin-coated vesicles. In addition, several types of non-clathrin-coated vesicles have been identified but their specific functions remain to be characterized.

Biochemical characterization of clathrin-coated vesicles revealed that their major coat components are ►clathrin and various types of adaptor complexes. Clathrin assembles in triskelions that consist of three heavy chains of approximately 190 kDa and three light chains of 30–40 kDa. Four types of adaptor complexes have been identified to date, AP-1, AP-2, AP-3 and AP-4 (AP for adaptor protein). Whereas AP-1, AP-3 and AP-4 mediate sorting events at the TGN and/or endosomes, AP-2 is involved in endocytosis at the plasma membrane. Each adaptor complex is a heterotetrameric protein complex, and the term ‘adaplin’ was extended to all subunits of these complexes. One complex is composed of two large adaptins (one each of $\gamma/\alpha/\delta/\epsilon$ and $\beta 1$ –4, respectively, 90–130 kDa), one medium adaptin ($\mu 1$ –4, <50 kDa), and one small adaptin ($\sigma 1$ –4, <20 kDa). In contrast to AP-1, AP-2 and AP-3, which interact directly with clathrin and are part of the clathrin-coated vesicles, AP-4 seems to be involved in budding of a certain type of non-clathrin-coated vesicles at the TGN.

A pre-requisite for clathrin-coat assembly is the recruitment to the membrane of an adaptor complex. Similar to what has been observed for the recruitment of coatamer to Golgi membranes, adaptor binding is dependent on the presence of ARF-GTP. However, in contrast to COPI vesicle formation, ARF-GTP is suggested to act in a process before budding and not as a stoichiometric coat component. Other differences between COP-coated and clathrin-coated vesicles concern their uncoating mechanism. Disassembly of clathrin-coated vesicles is believed to depend on the chaperone HSC 70 and on auxilin.

Membrane Proteins in Vesicle Formation and Cargo Selection

Formation of vesicles is likely to require interaction with the soluble coat components of cytoplasmic domains of certain integral membrane proteins that may serve as

coat receptors. Likewise, interaction of cytoplasmic domains of membrane cargo proteins with coat components may result in their selective packaging in a certain type of transport vesicle. Sorting of soluble cargo requires involvement of transmembrane receptors, which may couple sort in the lumen of an organelle to coat assembly at the cytoplasmic surface. The expected properties of a transmembrane cargo receptor include one or more transmembrane domains, a luminal domain able to interact with cargo species, and a cytoplasmic domain that interacts with coat subunits. Further, such proteins must cycle between the donor and the acceptor organelle. Coupling of coat assembly and cargo selection is best understood for clathrin-coated vesicles. The best known example for a cargo/coat-receptor is the ►mannose 6-phosphate receptor of TGN-derived clathrin-coated vesicles. On the luminal side, the receptor recognizes a mannose 6-phosphate signal of lysosomal hydrolases. On the cytoplasmic face, the receptor tail interacts with AP-1, thus initiating coat assembly. Sorting of membrane proteins into TGN-derived clathrin-coated vesicles has been shown to depend on a tyrosine-based motif (YXXF, where F can be replaced by a bulky hydrophobic amino acid) similar to the signal established for internalization via AP-2 at the plasma membrane. Sorting of membrane cargo into COPII pre-budding complexes was described for several proteins, and is believed to be mediated via an interaction with the Sec23p complex. For sorting of soluble cargo into retrograde COPI-coated vesicles, two types of membrane proteins are known to date that fulfill the criteria for cargo/coat receptors. One is the ►KDEL receptor, a multi-spanning membrane protein that mainly localizes to the Golgi and recognizes a carboxy-terminal tetrapeptide (KDEL) of soluble luminal proteins. The KDEL-sequence has been shown to serve as a retrieval signal of soluble proteins that have escaped from the ER. Another type of vesicular transmembrane proteins is referred to the p24 family, some members of which have been found in both COPII- and COPI-coated vesicles. These type I membrane proteins share a common structural organization: a large luminal domain, one-membrane spanning domain, and a short cytoplasmic domain with two conserved motifs: a di-phenylalanine motif and a di-basic motif at the extreme C-terminus. Two members of the p24 family, p23 and p24, were the first transmembrane proteins to be identified in COPI-coated vesicles. Both proteins are abundant in Golgi membranes and are concentrated into Golgi-derived COPI-coated vesicles where they are present in approximately stoichiometric amounts relative to ARF1 and coatamer. These proteins bind directly to coatamer and cycle within the early secretory pathway. It was also shown that both proteins interact with COPII *in vitro* and that they form hetero-oligomeric complexes with various members of the

►p24-family (p25, p26 and p27). Reconstitution of COPI-coated vesicles from chemically defined liposomes revealed that p23 is part of the minimal machinery for budding of COPI-coated vesicles. The data presently available make the p24 proteins strong candidates for coat receptors.

Mechanism In Vesicle Fusion

Once a coated vesicle is formed, delivery of its cargo to the correct destination depends on the accurate and specific recognition of the target membrane and subsequent fusion. A conceptual framework for explaining how transport vesicles dock to an acceptor membrane was formulated in the ►SNARE hypothesis. This hypothesis states that the specificity of vesicle targeting is generated by highly specific complexes that form between membrane proteins of the vesicle (v-SNAREs) and membrane proteins of the acceptor membrane (t-SNAREs). Structural and biophysical studies have shown that the cytoplasmic domains of v- and t-SNAREs form α -helical bundles with high thermal stability. During pairing of cognate v- and t-SNAREs the cytoplasmic core domains build a rod-shaped coiled-coil complex that is composed of four α -helices. Formation of this stable complex pulls the vesicle and the target membrane in close proximity, thus providing the driving force for fusion. Thus, the SNARE-complex represents the minimal machinery needed for the fusion process, as has been shown by reconstitution in liposomes with defined proteins. In addition, specificity of SNARE-pairing is controlled by so-called tethering proteins and another class of GTP-binding proteins, the Rabs.

Pharmacological Intervention

Progress during the last few years to understanding the mechanisms that underlie transport of proteins and lipids within the secretory pathway, as well as the sequencing of the human genome revealed that many human diseases are due to defects in intracellular trafficking, like, e.g. I-cell disease and familial hypercholesterolaemia. Obviously, a better understanding at the molecular level of intracellular transport within the secretory pathway, combined with increasing knowledge of the molecular basis of 'transport diseases' will open ways to therapeutic interventions.

- Protein Trafficking and Quality Control
- Exocytosis

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Intrathecal Application

Administration of a drug into the cerebrospinal fluid (liquor cerebrospinalis), by performing either a lumbar or a ventricular injection.

- Antimetabolites
- Intrathecal Space

Intrathecal Space

The intrathecal space is located between the arachnoid and the pia mater of the spinal cord. It contains the cerebrospinal fluid, spinal nerves and blood vessels.

- Intrathecal Application

Intrinsic Efficacy

Efficacy in drug receptor theory was originally defined by R.P. Stephenson (1956) as a dimensionless quantity of excitation given to a receptor to induce a response. Furchgott (1966) refined the definition to intrinsic efficacy as the quantal unit of stimulus given to a single receptor by an agonist. Subsequent research indicates that receptors mediate behaviors in addition to physiological response (some independent of ligands such as ► [constitutive receptor activity](#)). Hence a more encompassing definition of intrinsic efficacy is the property of a ligand that causes the receptor to change its behavior toward the host cell.

- Drug–receptor Interaction

Intron

Noncoding region only found in eukaryotic genes. During transcription in the nucleus, RNA is generated from both introns and ► [exons](#) – the coding regions – but the introns are excised by RNA splicing when mRNA is produced. During this process, the splicing machinery recognizes defined splice sites in the RNA sequence.

- Exon

Inverse Agonist

Receptor ligands that induce cellular reactions opposite to those caused by natural agonists, i.e., hormones or neurotransmitters, are called inverse agonists. These compounds show “negative intrinsic activity” on their receptor. Inverse agonists are assumed to stabilize the receptor in the inactive form. They can be used for silencing constitutively active receptors. A majority of antagonists not only preoccupy the agonist binding site on a receptor without affecting its equilibrium between the active and inactive states, but rather stabilize the inactive receptor conformation, thereby acting as inverse agonists.

- Transmembrane Signaling
- Drug–receptor Interaction
- G-protein-coupled Receptors
- Histaminergic System
- Adenosine Receptors

Inward Rectification

Inward Rectification refers to decreased conductance upon membrane depolarization. In classical inward rectifier K^+ channels, rectification is “strong” and currents rapidly decline at membrane potentials positive to the reversal potential, in contrast to other ► [Kir channels](#) in which rectification is “weak” and currents decline only gradually at potentials positive to the reversal potential.

- Inward Rectifier K^+ Channels

Inward Rectifier K⁺ Channels

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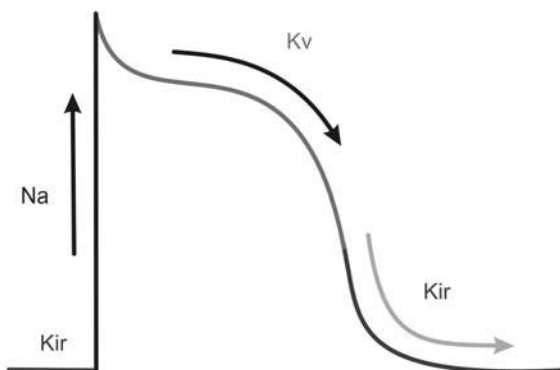
Synonyms

Inwardly rectifying K⁺ channels; Anomalous rectifiers;
►Kir channels

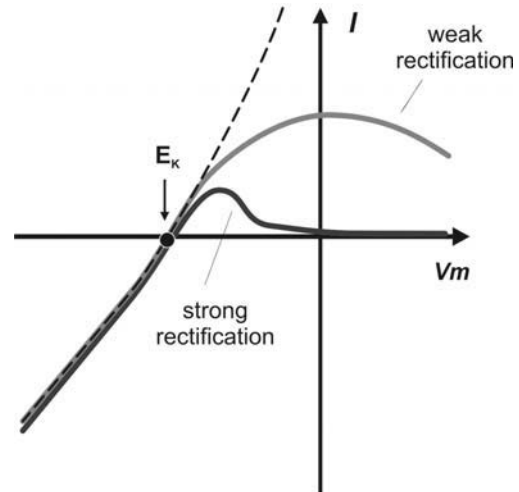
Definitions

Inward Rectifier Potassium Channels or ►Kir Channels are a class of potassium channels generated by ►tetrameric arrangement of one-pore/two-transmembrane helix (1P/2TM) protein subunits, often associated with additional beta-subunits. ►Kir channels modulate cell ►excitability, being involved in ►repolarization of ►action potentials (see Fig. 1), setting the ►resting potential (see Fig. 1) of the cell, and contributing to ►potassium homeostasis.

Basic characteristics: Inward Rectifier Potassium Channels or ►Kir Channels are a class of potassium channels generated by ►Inward Rectification, which decreased conductance upon depolarization. In classical inward rectifiers, rectification is “strong” and currents rapidly decline at voltages positive to the reversal potential (see Fig. 2). In other ►Kir channels, rectification is “weak” and currents decline only gradually at voltages positive to the reversal potential (see Fig. 2).



Inward Rectifier K⁺ Channels. Figure 1 The role of inward rectifier (Kir) channels in cardiac action potentials. Depolarization is generated and maintained by Na and Ca currents (I_{Na} , I_{Ca}). Voltage-gated K currents (Kv) and Kir channels contribute to repolarization and maintenance of a negative resting potential.



Inward Rectifier K⁺ Channels. Figure 2 High [K⁺] inside cells relative to outside results in “normal” rectification, whereby outward (positive by convention) potassium currents (I) when cells are depolarized (V_m is positive relative to E_K), are bigger than inward (negative) currents at hyperpolarized (negative) voltages. Inward or anomalous rectifiers show “strong” or “weak” inward rectification whereby outward currents are smaller than inward currents.

Basic Characteristics

Seven subfamilies of eukaryotic ►Kir channels, each sharing ~60% amino acid identity between individual members within each subfamily and ~40% identity between subfamilies, are known [1]. In addition, multiple prokaryotic ►Kir channels (Kirbac1.1–9) are now being identified in bacterial genomes. We will focus on the eukaryotic channels.

Kir1 subfamily. Kir1.1 (ROMK1, gene *KCNJ1*) [1] encodes a “weak” (see below) inward rectifier and is expressed predominantly in the kidney. Alternate splicing at the 5' end also generates multiple Kir1.1 splice variants, Kir1.1a (ROMK2) through Kir1.1f (ROMK6), some of which are ubiquitously expressed in various tissues, including kidney, brain, heart, liver, pancreas, and skeletal muscle. In the kidney the renal Kir1.1 channels control salt reabsorption.

Kir2 subfamily. Four distinct Kir2 subfamily members (Kir2.1–Kir2.4; *KCNJ2*, *KCNJ12*, *KCNJ4*, *KCNJ14*) have been cloned to date [1], all encoding classical “strong” inward rectifiers that differ in single channel conductance and in sensitivity to phosphorylation and other second messengers. Members of Kir2 subfamily are highly expressed in the heart and skeletal muscle, but also found in a number of other tissues (e.g., nervous system). Kir2 subunits are the key players in cardiac inward rectifier current ► I_{K1} present in atrial and ventricular myocytes. Kir2.1 is a predominant isoform in all species while Kir2.2 and Kir2.3 may also

contribute. Kir2.4 is believed to be restricted to neuronal cells in cardiac tissue.

Kir3 subfamily. Four members of the Kir3 subfamily (Kir3.1–Kir3.4: *KCNJ3*, *KCNJ6*, *KCNJ9*, *KCNJ5*) express G-protein activated “strong” inward rectifier **K channels** (**GIRK channels**) underlying G-protein-coupled receptor-activated currents in heart, brain, and endocrine tissues. Functional channels require coassembly of two different subunits (Kir3.1 and Kir3.4). Several studies have provided evidence for a promiscuous coupling between the various members of the Kir3 subfamily.

Kir4 and Kir5 subfamilies. These subfamilies of **Kir channels** (Kir4.1 and Kir4.2, *KCNJ10*, *KCNJ15*, Kir5.1, *KCNJ16*) are abundantly expressed in brain and kidney. Kir4.1 forms “weak” inward rectifier **K channels** when expressed alone while Kir5.1 does not form homomeric channels. Coexpression of Kir4.1 and Kir5.1 subunits results in formation of channels with properties significantly different from those of homomeric Kir4.1 channels. Little is known about the physiological role of channels derived from Kir4 and Kir5 subfamilies, although they are clearly involved in generating glial **Kir channels**. The activity of Kir5.1/Kir4.1 heteromeric channels is very sensitive to intracellular pH and this property is conferred predominantly by the Kir5.1 subunits.

Kir6 subfamily. Two members (Kir6.1, Kir6.2, *KCNJ8*, *KCNJ11*) of this subfamily encode ATP-sensitive K⁺ channels (K_{ATP}) that are inhibited by intracellular ATP and activated by ADP, thereby coupling cell metabolism to **excitability** [2]. K_{ATP} channels are found in many tissues including ventricular and atrial cells (Kir6.2 in the cell membrane, Kir6.1 presumably in mitochondrial membranes). Functional expression of active channels requires coexpression of Kir6.x subunits with a sulfonylurea receptor (SUR1 or SUR2). K_{ATP} channels display “weak” rectification, allowing substantial outward current to flow at positive potentials, and thus causing **action potential** shortening, or loss of **excitability**, when activated, for example, during metabolic inhibition [2].

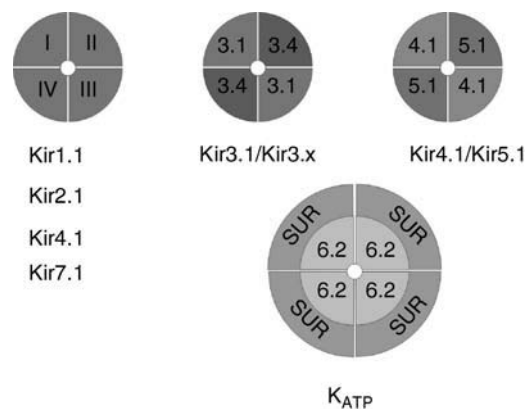
Kir7 subfamily. The only known member (Kir7.1, *KCNJ13*) is primarily expressed in brain, retinal pigment epithelium, but it is also found in a variety of other tissues, including kidney and intestine. This weakly rectifying channel has an apparently very low single channel conductance, very shallow dependence on external K⁺ and is virtually insensitive to intracellular Mg²⁺. The exact physiological role of this channel is obscure although suggestions are made that Kir7.1 may contribute to K⁺ recycling processes.

KirBac family. Multiple structurally related channel subunits are now being identified in bacteria, and by crystallization these provide templates for understanding Kir channel structure [3]. Functional roles in bacteria are unknown.

Molecular Structure of Kir Channels

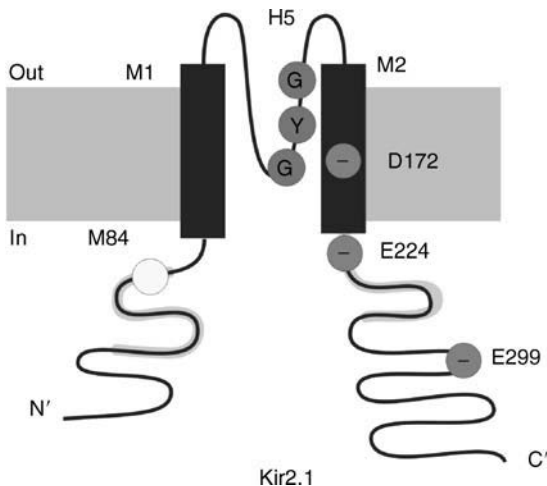
All **Kir channels** are **tetrameric** proteins (see Fig. 3) of one-pore/two-transmembrane (1P/2TM) domain subunits which equally contribute to the formation of highly selective K⁺ channels. Most **Kir channels** can be assembled in functional homotetramers while some require heteromeric assembly (see Fig. 3). For example, functional GIRK channels underlying I_{K_{ACh}} (Acetylcholine-activated) current in atria are heteromultimers of two members of Kir3 subfamily: Kir3.1 and Kir3.4.

It should be noted that not all **Kir channels** rectify strongly enough to fit the definition of classical rectification, such as observed in **cardiac I_{K1}** channels. A feature of all K⁺ selective channels is the signature G-Y-G sequence within the pore loop (P-loop) (see Fig. 4) that acts as a filter, to confer high selectivity to K⁺ ions, and also contributes to single channel conductance and kinetics. Two transmembrane domains with N'- and C'-termini facing the cytoplasm flank the P-loop (see Fig. 4). The assembly of the Kir2.1 channel is supported by the presence of intrasubunit disulfide bonds between highly conserved cysteine residues (at positions 122 and 154) which are absolutely required for proper channel folding, although disruption of the bond with reducing agents does not disrupt channel activity once the channel is already assembled. Scanning cysteine mutagenesis studies of relatively large stretches of N- and C-termini of Kir2.1 reveal that nearly half of them are water accessible, and potentially facing the pore, in addition to residues in the second transmembrane region. There is accumulating evidence that the overall structure of the core region of all **Kir channels** is very similar to that of the recently crystallized bacterial KirBac1.1 channel [3]. Crystal structures of the cytoplasmic domains of eukaryotic Kir2.1 and Kir3.1 channels have also been



Inward Rectifier K⁺ Channels. Figure 3

Kir channels may be homo- or hetero-tetrameric complexes, in some cases in tight association with beta-subunits (e.g., the K_{ATP} channel). SUR – sulfonylurea receptor.

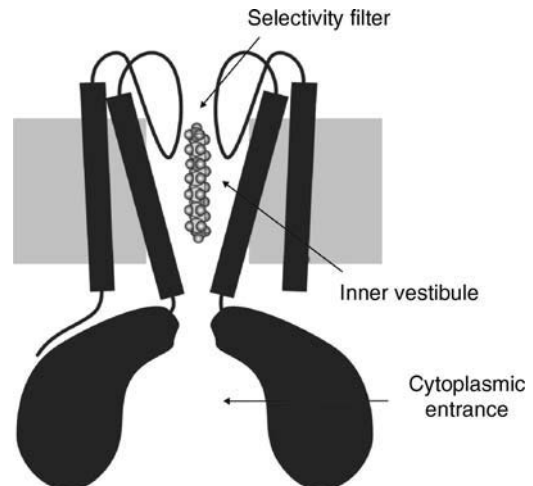


Inward Rectifier K⁺ Channels. Figure 4 Kir channel subunits consist of two transmembrane domains (M1, M2), separated by a pore loop (P-loop) that contains the signature K⁺-selectivity sequence (-GYG-), as well as extended cytoplasmic N'- and C-termini. Several residues (indicated) have been implicated in causing rectification (see text).

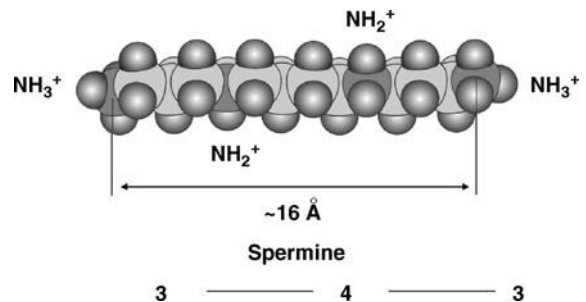
resolved, again showing the presence of wide intracellular vestibule, large enough to accept multiple permeating and blocking ions. Recent results suggest that cytoplasmic domains of ►Kir channels may undergo structural changes to modulate gating and inward rectification.

The Mechanism of Strong Inward Rectification

Inward or “anomalous” rectification of potassium permeability refers to increases of potassium conductance under hyperpolarization and decreases under depolarization, the effect opposite to that of “normal” outward or delayed rectification that is seen in voltage-gated K⁺ channels (see Fig. 2). Classical ►inward rectification is so strong that only small currents can be measured in the outward direction at voltages positive to the K⁺ reversal potential (E_K) while large inward currents can be easily observed negative to it. This strongly voltage-dependent rectification also strongly depends on the concentration of external K⁺ ($[K_{OUT}]$), such that increasing K_{OUT} relieves the rectification, so that the mid-point voltage of rectification shifts nearly perfectly with corresponding change in E_K . It is now established that strong ►inward rectification results primarily from voltage-dependent block by intracellular organic cations called ►polyamines [1]. Of the ►polyamines, ►spermine (see Figs. 5 and 6) and ►spermidine are the most potent inducers of rectification although contributions of putrescine and of Mg^{2+} ions are also important. Both the steady-state and the kinetic properties of rectification result from the combined action of ►polyamines and Mg^{2+} ions. Micromolar concentrations of free ►spermine and ►spermidine are sufficient



Inward Rectifier K⁺ Channels. Figure 5 Proposed three-dimensional arrangement of the transmembrane region of Kir channels. Two of four subunits are indicated. The pore consists of a selectivity filter close to the outside part of the membrane, a central inner vestibule, and a cytoplasmic entrance. Spermine may block in the inner cavity or in the selectivity filter. Large intracellular vestibule where polyamines may also block the channel is not shown.



Inward Rectifier K⁺ Channels. Figure 6 Spermine (Amino-propyl-amino-butyl-amino-propyl amine) is a long linear naturally occurring polyamine containing four nearly equally spaced positively charged (at normal pH) amines. Although ~16 Å long, the molecule is only about 3 Å wide, similar to a dehydrated potassium ion.

to reproduce the degree of rectification seen in native cells [1]. Importantly, in cells the total ►polyamine levels are quite large (up to 10 mM), but most of polyamines are bound to various intracellular targets such as RNA, DNA, ATP and other primarily phosphate-containing molecules.

The degree of rectification varies greatly among members of the Kir superfamily and is fundamental to their respective functional roles. Kir2 and Kir3 encode classical “strong” inward rectifier channels, while other members encode channels with variably “milder” or

“weaker” rectification (see Fig. 2). For example, because of mild rectification of the K_{ATP} channel, its activation causes considerable shortening of the cardiac ▶action potential, thus reducing entry of Ca²⁺ through voltage-dependent Ca²⁺ channels and hence conserving ATP under conditions of metabolic stress. Conversely, the strong ▶inward rectification of Kir2.1 channels underlying I_{K1} current in the heart results in very small currents flowing through these channels during depolarization phase of ▶action potential while increased conductance around ▶resting potential leads to its stabilization [4].

Structural Elements of Inward Rectification

Each Kir subunit consists of two transmembrane helices (M1, M2), with a pore-forming selectivity filter linking them, and cytoplasmic N'- and C'-termini (see Fig. 4 and [3]). Based on the crystal structure of the related KirBac1.1 channel [3], the channel is proposed to be formed as a ▶tetrameric arrangement of these subunits, surrounding an external selectivity filter, an inner vestibule and a cytoplasmic entrance to the pore (see Fig. 3). Aspartate 172 located in the M2 region of Kir2.1 was the first residue implicated in the classical rectification of these channels and is sometimes referred to as the “▶rectification controller.” Later, a number of other residues in the C terminus of Kir2.1 were also shown to contribute to ▶polyamine-induced ▶rectification. Neutralization of some of these negatively charged amino acids may transform the strongly rectifying Kir2.1 channel into one that is nearly insensitive to blockage by ▶polyamines and Mg²⁺. Cytoplasmic residues located in the N terminus of Kir2.1, such as M84, may also be important for channel blockage by intracellular cations. At present the question about the exact location(s) of the binding sites for ▶polyamines remains controversial. Probably one ▶spermine molecule is required to completely block the channel, either within the inner vestibule, or perhaps within the selectivity filter itself. There have been reports that the Kir pore region may be “unprecedentedly wide” allowing simultaneous binding of three Mg²⁺ ions or three ▶polyamine molecules. Reconciliation of conflicting interpretations can probably be achieved by assuming that gating of ▶Kir channels may involve substantial conformational changes, including helix rotations and translational movements similar to those observed in the related bacterial KcsA channel [3]. The selectivity filter of Kir2.1 is probably the best candidate for the binding of polyamines. Despite the relatively large size of the ▶spermine molecule (~20 Å long) (see Fig. 3), its diameter is close to that of a dehydrated K⁺ ion, thus potentially allowing its head amine group to “squeeze” into the selectivity filter and block ion flow (see Fig. 6). ▶Polyamines may also permeate ▶Kir channels, and can clearly permeate, as well as block, other nonselective cationic channels, consistent with such an interpretation.

Known Diseases (Channelopathies) Resulting from ▶Kir Channel Mutations

Multiple ▶channelopathies resulting from mutations in ▶Kir channels are known.

Kir1.1. Bartter Syndrome. Several mutations in the core region as well as in the N' and C' terminus of Kir1.1 are found in patients with hyperprostaglandin E syndrome (HPS; renal disorder resulting from impairment of tubular reabsorption), an antenatal form of Bartter syndrome. Some of these mutations result in the loss of function of Kir1.1 channels causing impaired renal K⁺ secretion and NaCl reabsorption.

Kir2.1. Andersen's syndrome. Dominantly inherited Long QT syndrome (LQT), a disorder of cardiac ▶action potential repolarization is usually assigned to mutations in cardiac Na⁺ or voltage-gated K⁺ channels. Recently, it has been found that multiple mutations in *Kir2.1* cause Andersen's syndrome (LQT7), a rare disease characterized by periodic paralysis, cardiac arrhythmias, and dysmorphic features. Mutations in *Kir2.1* associated with Andersen's syndrome were found to cause dominant negative suppression of the wild type Kir2.1 channels when expressed in *Xenopus* oocytes or cultured cell lines thus mimicking the effects of the *Kir2.1* gene knock-out which is characterized by prolonged QT interval. Alternatively, mutation in the “▶rectification controller” of Kir2.1 channel (D172N mutation) was recently identified leading to a new form of Short QT syndrome (SQT3), which is characterized by a unique ECG phenotype. Another gain-of-function mutation in human *Kir2.1* gene (V93I) was also found to underlie a **familial atrial fibrillation**.

Kir3.2 Weaver mouse. A mutant mouse with cerebellar degeneration and motor dysfunction resulting from a serine for glycine substitution in the -GYG- sequence of the K selectivity filter of Kir3.2. G-protein activated K conductances are abolished in the cerebellar neurons, leading to Ca²⁺ overload and cell death.

Kir6.2/SUR Persistent Hyperinsulinemic Hypoglycemia of Infancy (PHHI) and Neonatal Diabetes. Lowered blood glucose normally causes decreased ATP/ADP ratios in the pancreatic islet β-cells, causing the opening of K_{ATP} channels, hyperpolarization, inhibition of Ca²⁺ entry and cessation of insulin secretion. In PHHI, K_{ATP} channel mutations lead to abolition of activity and hence maintained depolarization and maintained Ca²⁺ entry and insulin secretion. Many mutations in the SUR subunit abolish ADP activation of channels, but point mutations in Kir6.2 are implicated in abolition of channel activity in some cases. Conversely, K_{ATP} channel mutations that lead to increased activity and hence maintained hyperpolarization and failure of Ca²⁺ entry block insulin secretion and cause neonatal diabetes. Many mutations in the Kir6.2 and SUR subunits have now been identified, and

this has led to a change in therapy for the disease, from injected insulin to sulfonylurea pills [2].

Drugs

Voltage-dependent block by external Ba^{2+} and Cs^{+} ions, yet insensitivity to the Kv channel blocker tetraethyl ammonium (TEA) have been the classical tools to examine ►Kir channel activity. Recently, the honey-bee venom tertiapin has been found to be an effective blocker of certain (Kir1, Kir3) inward rectifier subfamily members. Tight association of Kir6 family members with SUR subunits endows K_{ATP} channels with a rich pharmacology: channel activity is very specifically inhibited by sulfonylurea drugs such as tolbutamide and glibenclamide, and is activated by a broad class of “potassium channel opening” (KCO) drugs such as pinacidil and diazoxide. A potent antiarrhythmic drug ►RP58866 and its active enantiomer RP62719 (known as ►Terikalant) has been shown to block IK_{ACh} (encoded by Kir3.x members) and ► I_{K1} (encoded by Kir2.x members) currents in the heart in the low micromolar range. Unfortunately, neither agent discriminates well between Kir and other ►K channels (e.g., underlying ►Ikr) and thus has not received much attention as a selective blocker for ►Kir channels.

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Ion Channels

Ion channels are proteins which span the plasma membrane and can be opened by transmembrane voltage changes (voltage-dependent ion currents) or by binding of a neurotransmitter. Ion channels which are selective for Na^{+} or Ca^{2+} ions cause excitation, ion channels with selectivity for Cl^{-} or K^{+} usually cause inhibition of cells.

Ion channels are often multimeric and are regulated by a wide variety of mechanisms (e.g., ligand binding, voltage changes, phosphorylation).

- Voltage-dependent Na^{+} Channels
- Voltage-dependent Ca^{2+} Channels
- K^{+} Channels
- Non-selective Cation Channels
- TRP Channels
- Cl^{-} Channels and $\text{Cl}^{-}/\text{H}^{+}$ Exchangers
- Epithelial Na^{+} Channel
- Nicotinic Receptor
- Ionotropic Glutamate Receptors
- Purinergic System
- Serotonergic System
- Glycine Receptor
- GABAergic System

Ionic Contrast Media

Ionic contrast media are triiodobenzene derivatives carrying a negative electrical charge, water soluble only as sodium or meglumine (an organic cation similar to glucosamine) salts.

- X-ray Contrast Agents and Molecular Imaging

Iodide

- Antithyroid Drugs

Iodine

- Antithyroid Drugs

Ionotropic Glutamate Receptors

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Definition

Most neurons in the central nervous system are stimulated by L-►glutamate, the major excitatory amino acid in the brain. The postsynaptic actions of

this neurotransmitter are mediated by two categories of glutamate receptors: the ionotropic glutamate receptors that directly gate channels and the metabotropic glutamate receptors that indirectly gate channels via second messengers. The ionotropic glutamate receptors can be further subdivided into N-methyl-D-aspartate (NMDA) receptors (►NMDA receptor) and non-NMDA receptors, with AMPA (L- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and ►kainate receptors constituting the latter group [1, 2]. These three major subtypes of ionotropic glutamate receptors are named according to their selective agonists NMDA, AMPA and kainate. These receptors mediate most of the fast excitatory neurotransmission in the brain.

NMDA- and AMPA receptors (►AMPA receptor) are colocalized in the postsynaptic membrane of excitatory synapses. Glutamate released from presynaptic terminals binds to both types of receptors. Upon activation by glutamate, AMPA receptors generate the large early component of an excitatory postsynaptic current (EPSC) because of their rapid ►gating kinetics. This synaptic current is mainly generated by Na^+ and K^+ , but not Ca^{2+} ions, since AMPA receptors in excitatory neurons are usually impermeable to Ca^{2+} . In contrast to this, NMDA receptors have relatively slow gating kinetics and contribute to the late component of the EPSC. The NMDA receptors are permeable for Na^+ and K^+ as well as Ca^{2+} ions and require extracellular glycine as a cofactor for activation. Most importantly, this activation is not only dependent on the presence of the agonist, but also depends on the membrane voltage. Therefore, the receptor is not involved in generation of the early component of the EPSC after the binding of glutamate. The channel gets activated only when the binding of glutamate plus the coagonist glycine and depolarization of the membrane occur at the same time. This voltage dependence is due to extracellular Mg^{2+} . At resting membrane potential extracellular Mg^{2+} binds tightly to a site in the pore and blocks the channel. Only at depolarized membrane potentials, which are generated by the AMPA receptors, Mg^{2+} is expelled from the pore by electrostatic repulsion, thereby lifting the Mg^{2+} block and Na^+ , K^+ and Ca^{2+} cations can cross the channel. Most neurons express both NMDA and AMPA receptors. However, the EPSC generated at resting membrane potential is mainly generated by activation of AMPA receptors. NMDA receptors do not contribute significantly to the EPSC since at membrane resting potential Mg^{2+} is blocking the channel. Only with increasing membrane depolarization NMDA receptors contribute to the EPSC, since Mg^{2+} is removed and ions flow through the channel.

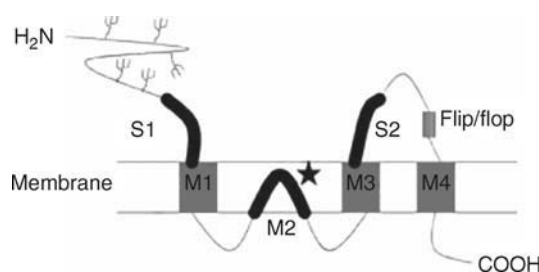
Whereas the role of AMPA and NMDA receptors in fast synaptic transmission is well characterized, only few examples demonstrating synaptic responses due to kainate receptor activation are known so far.

Basic Characteristics

Sequence homologies and sometimes similarity in gene structure suggest a common evolutionary origin for all ionotropic glutamate receptors. The cloning of the ionotropic glutamate receptor genes revealed that NMDA, AMPA and kainate receptor subunits are encoded by at least six gene families (a single family for AMPA receptors, two for kainate, and two for NMDA receptors).

The primary structure of the cloned receptor subunit genes revealed prominent structural similarities between NMDA, AMPA and kainate receptors (Fig. 1). The transmembrane topology of these receptors is very different from that of other ionotropic channels. Members of other ionotropic receptor families, which are activated by acetylcholine, GABA (γ -aminobutyric acid) or glycine, contain four transmembrane segments. In contrast to these, the ionotropic glutamate receptor subunits contain three transmembrane domains (M1, M3 and M4) and a cytoplasm-facing membrane loop (M2), which connects the transmembrane domains M1 and M3 and forms the channel pore. Key amino acids in the M2 segment are responsible for the differences in ion selectivity displayed by the various receptor subunits. Two regions of the glutamate receptors are extracellularly located: the amino-terminus and the region between M3 and M4, which are involved in building the ligand-binding site. The carboxy-terminal part after M4 reaches into the cytoplasm of the cell and is important for intracellular modifications like phosphorylation or interaction with cytoplasmic proteins.

Both AMPA and NMDA receptors are multimeric, probably tetrameric, assemblies of various molecularly distinct subunits, giving rise to large receptor diversity. For AMPA receptors this is achieved by assembling the



Ionotropic Glutamate Receptors. Figure 1 Schematic structure of a ionotropic glutamate receptor subunit. The three transmembrane domains M1, M3 and M4 are shown as gray boxes, the membrane loop M2 forms the channel pore. The star indicates the position of key amino acids regulating the ion selectivity, e.g., the Q/R for AMPA, N for NMDA receptors. S1 and S2 designate the two ligand-binding domains. The alternatively spliced flip/flop exon occurs in AMPA receptors and is located extracellularly. Potential glycosylation sites are shown as trees in the N-terminal region.

receptor from four types of subunits, termed GluR (glutamate receptor)-A, -B, -C and -D (in an alternative nomenclature GluR1, GluR2, GluR3 and GluR4), with additional molecular diversity generated by ►RNA editing at the glutamine/arginine (Q/R) and arginine/glycine (R/G) site as well as alternative splicing of the flip-flop module. NMDA receptors are assembled from NR1 subunits and NR2A, B, C or D subunits. Several splice variants have been identified for the NR1 subunits, and the NR3 subunit, that has been identified recently, appears to be part of a glycine-gated channel. Kainate receptors are formed by subunits that can be divided into two subfamilies: the first subfamily contains the subunits GluR5, GluR6 and GluR7, whereas the second subfamily comprises KA1 and KA2. All subunits of the first group can form functional channels, whereas the subunits of the second group do not form homomeric channels. Several splice variants have been identified for the GluR5 and GluR7 subunits. This large number of combinatorial possibilities accounts for a considerable molecular diversity of glutamate receptor channels.

Recombinant expression of the AMPA receptor subunits has shown that homomeric AMPA receptors assembled from different subunits are different in a number of characteristics. These are mostly determined by the GluR-B subunit. Receptors formed from GluR-B subunits show low Ca^{2+} permeability whereas receptors assembled from GluR-A, -C and -D subunits are highly Ca^{2+} permeable. A single amino acid difference in the pore-forming segment M2 has been identified as the molecular determinant of the subunit-specific difference in Ca^{2+} permeability. Whereas the GluR-B subunit contains a positively charged arginine (R), the GluR-A, -C and -D subunits contain a neutral glutamine residue (Q) at this position. This amino acid exchange at the Q/R site is sufficient to abolish the permeability to Ca^{2+} , possibly through electrostatic repulsion, indicating that the Q/R site is the main determinant of the Ca^{2+} permeability in recombinant AMPA receptors. In native AMPA receptors, which are heteromeric assemblies, the Ca^{2+} permeability of recombinant receptors is determined by the GluR-B(R) subunits. Therefore strong differences in Ca^{2+} permeability can be observed between different cell types [1]. Excitatory neurons strongly express GluR-B(R), which leads to the formation of Ca^{2+} impermeable AMPA receptors. In contrast, inhibitory neurons express the GluR-B(R) subunit to a low extent and therefore contain AMPA receptors that are highly permeable to Ca^{2+} ions. The subunit composition and electrical properties of AMPA receptors can also vary in a synapse-specific manner within individual cells [6].

Interestingly, the genomic sequences of all AMPA receptor subunits contain a Q codon for this position and the R codon is selectively introduced into the GluR-B pre-mRNA by RNA editing [3]. Additionally, the Q/R site is a critical determinant of current

►rectification and single-channel conductance. In recombinant AMPA receptors, GluR-B(R)-containing channels show a linear current–voltage relation (I – V), whereas it is inwardly or doubly rectifying in AMPA receptors that only contain GluR-A, -C or -D subunits. It has been shown that this current rectification is due to a voltage-dependent block by intracellular ►polyamines, such as spermine or spermidine, which block GluR-B(R)-free but not GluR-B(R)-containing AMPA receptors. The physiological significance of this block is unknown, but it may act similar to the extracellular Mg^{2+} block of NMDA receptor channels. But whereas a single GluR-B(R) subunit per channel is possibly sufficient to abolish Ca^{2+} permeability, several GluR-B(R) subunits are necessary to suppress polyamine sensitivity. Another basic property of the AMPA receptor that is determined by the Q/R site is the single-channel conductance. If the receptor contains a GluR-B(R) subunit, it has a two- to three-fold lower single-channel conductance. Each AMPA receptor subunit exists as either a flip or a flop variant, which is determined by mutually exclusive splicing of an exon encoding a domain of 38 amino acids. Alternative splicing and editing of the GluR-B, -C and -D subunit at the R/G site influences receptor deactivation, desensitization and recovery from ►desensitization. The flip-flop module as well as the R/G site is probably located in the extracellular loop between M3 and M4, which is thought to be part of the agonist-binding site. This suggests relationships between agonist binding and gating properties of the receptor.

Functional NMDA receptors are heteromeric channels, which are composed of a principal NR1 subunit and modulatory NR2 subunits (NR2A, -B, -C and -D). They differ from AMPA receptors in several properties. Some of the most important properties are requirement for glycine for activation, the very high permeability for Ca^{2+} , the voltage-dependent block by extracellular Mg^{2+} and the slow gating kinetics. NMDA receptors require not only glutamate for activation, but also glycine as a coagonist. In the receptor the binding site for glutamate is generated by the NR2 subunit whereas the binding site for glycine is supplied by the NR1 subunit.

In contrast to AMPA receptors, NMDA receptor channels display a prominent Ca^{2+} permeability, which is largely independent of the subunit composition. It has been shown by mutational analysis that the Ca^{2+} permeability of recombinant NMDA receptors is dependent on a residue at a position equivalent to the Q/R site of AMPA subunits. Both NR1 and NR2 subunits contain an asparagine (N) residue at this position. Replacing this N with an R within the NR1 subunit led to the formation of NMDA receptors with a strongly reduced Ca^{2+} permeability, whereas exchanging N for Q in the NR2 subunit had only a small effect,

indicating that the N site of the NR1 subunit is the main determinant of the Ca^{2+} permeability of recombinant NMDA receptors. The situation is more complex for the other important property of the NMDA receptor, the extracellular Mg^{2+} block. Mutational analysis has revealed that the two residues downstream of the Q/R/N site are important for the control of the Mg^{2+} block. Furthermore, differences in Mg^{2+} sensitivity in the different NR1-NR2 combinations are generated at multiple locations throughout the subunit, including M1, the M1/M2 linker and the M4 segment.

Another characteristic distinguishing NMDA from AMPA receptors is the slow gating kinetics. Whereas recombinant AMPA receptors display deactivation time constants in the range of a few milliseconds, deactivation time constants for recombinant NMDA receptors are in the range of hundreds of milliseconds up to seconds, depending on which NR2 subunit is used to form the receptor. Furthermore it is worth mentioning that other factors than subunit composition can influence functional properties of both AMPA and NMDA receptors. Thus, there is growing evidence that *in vivo* receptor localisation (extrasynaptic versus synaptic) and auxiliary proteins and interactions with scaffolding complexes mediate and modulate signalling during neuronal development, synaptic plasticity and disease [7]. Desensitization of NMDA receptors is more complex than of AMPA receptors. At least three different forms have been described, all of which are mediated by the NR2 subunit.

The two kainate receptor subunits GluR5 and GluR6 undergo posttranscriptional modification similar to AMPA receptors. These two subunits also contain a Q/R site that is modified by RNA editing [3]. The Q/R site seems to have a strong influence on the functional properties of kainate receptors, similar to what has been observed in AMPA receptors. The GluR6(Q) subunit shows strong inward rectification whereas GluR6(R) does not rectify. And, like in AMPA receptors, the inward rectification of GluR6(Q) channels is most likely due to blocking of the channel by intracellular polyamines. But in contrast to AMPA receptors, where the GluR-B subunit is almost completely Q/R site-edited, significant proportions of the kainate subunits remain unedited. No editing occurs on the KA1 and KA2 subunit mRNAs, which carry a Q at the Q/R site. Editing of the Q/R site has a strong influence on single-channel conductance. Homomeric channels formed by edited subunits have a smaller single-channel conductance than homomeric channels formed by unedited subunits or heteromeric channels containing unedited subunits. Such a larger single-channel conductance for unedited receptors compared to edited receptors has also been observed for AMPA receptors. Desensitization and recovery from desensitization are different compared to other glutamate receptors. Whereas

desensitization of kainate receptors is very fast, recovery from desensitization is very slow.

Drugs

Changes in the physiological function of glutamate are thought to contribute to the pathogenesis of neurological diseases. Derivatives of quinoxaline-2,3-diones have long been shown to have an antagonistic effect on AMPA, kainate and NMDA receptor channels [4]. Representative examples of this group acting on AMPA receptors are 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX). In addition to being glycine-site antagonists for NMDA receptors they have also been shown to be competitive antagonists for AMPA and kainate receptors, with a much higher affinity for AMPA and kainate receptors than for the glycine binding-site of the NMDA receptors. The advent of these compounds allowed the study of the mechanism of fast synaptic transmission by AMPA receptors as well as of their potential in neuroprotection. But due to their nephrotoxic side effects and their poor solubility in water they are of limited therapeutic importance.

NMDA receptors play key roles not only in synaptic plasticity, but also in neurological diseases such as epilepsy and neurodegeneration. NMDA hypofunction has been proposed to underlie some behavioural symptoms in schizophrenia. Their ability to participate in excitotoxic events is due to the high Ca^{2+} permeability, their high affinity for glutamate and their relative lack of desensitization. This has prompted extensive research for selective NMDA receptor antagonists [4]. Different types of antagonists have been developed, in part acting on different parts of the receptor. Compounds such as dizocilpine (MK-801), aptiganel, phencyclidine and ketamine are activity-dependent channel blockers. They need channel opening in order to bind to and block the receptor. Therefore, they are noncompetitive antagonists. Antagonists of the glutamate- and glycine-recognition site have also been developed. Antagonists against the glycine-recognition site such as kynurenic acid are of special interest because glycine is a coagonist at the NMDA receptor, but glutamate acts as the neurotransmitter. In contrast, glycine plays a more modulatory role, since it is always present in the extracellular fluid. Therefore, these antagonists would limit the level of NMDA receptor activation, but still allow for a certain physiological activation. Another approach taken is the development of subunit-selective compounds. One compound of this group of antagonists is ifenprodil and its derivatives Ro 25-6981 and CP-101,606 (traxoprodil). It is selective for the NR2B subunit and has a much lower affinity on receptors containing NR2A, C or D subunits. The action of ifenprodil is not only subunit-specific, but also state-dependent. It binds to the activated and desensitized receptor with a higher affinity than to a

receptor without a bound ligand, in this way exerting a stronger blockade on receptors continuously activated in a disease state whereas leaving normal fast synaptic transmission largely unaffected. A recently developed competitive antagonist for the NR2A-subunit in NVP-AAM077 that has been used to study the contribution of the NR2A subunit in synaptic plasticity and neurological diseases [8].

Antagonists selective for kainate receptors are not available yet. The non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) blocks AMPA as well as kainate receptors. Nevertheless, compounds like GYKI 53655, which acts as a noncompetitive antagonist of AMPA receptors and completely blocks AMPA receptor function at certain concentrations at which no antagonistic effect on kainate receptors is discernible, has been used to demonstrate the kainate receptor-mediated currents in neurons.

- Table Appendix: Receptor Proteins
- Metabotropic Glutamate Receptors

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Ionotropic Receptors (Channel-linked)

A number of agonists can act through several receptor classes, e.g., ion channels and ►G-protein-coupled receptors. To set receptor subtypes permanently linked to ion channels (►ligand-gated ion channels) apart

from G-protein-coupled receptor subtypes the terms ionotropic versus metabotropic receptors, respectively, are used. Ionotropic receptors are known for acetylcholine, ATP, serotonin, GABA, glutamate, and glycine.

- Transmembrane Signaling
- Nicotinic Receptors
- Purinergic System
- Serotonergic System
- GABAergic System
- Ionotropic Glutamate Receptors
- Glycine Receptors
- Table Appendix: Receptor Problems

IP₃ Receptors

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Synonyms

Inositol 1,4,5-trisphosphate receptor

Definition

Inositol 1,4,5-trisphosphate (IP₃) receptors are intracellular cation channels. They are expressed in most cells and predominantly within the membranes of the endoplasmic reticulum. They mediate release of Ca²⁺ from intracellular stores by the many receptors that stimulate IP₃ formation.

Basic Mechanisms

Ca²⁺ Signalling

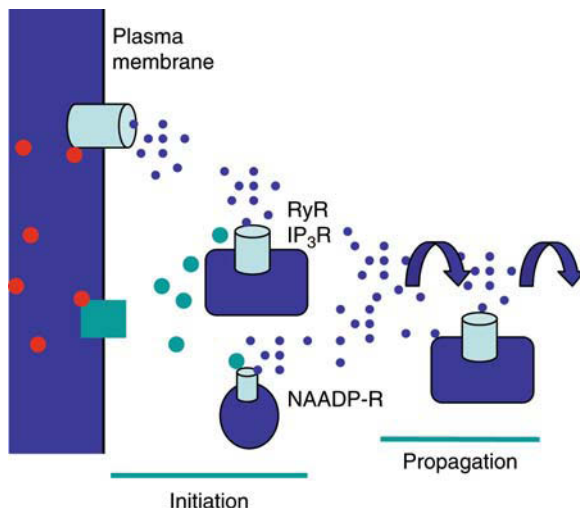
Ca²⁺ is an intracellular messenger used by all eukaryotic cells. It regulates cellular activities through every stage of the lifecycle, from fertilization, through growth and differentiation, to controlling the specialized behaviours of differentiated cells, and ultimately in controlling cell death [1]. The ability of cells actively to extrude Ca²⁺ from their cytosol – a property that probably evolved in parallel with the use of ATP as an energy currency – provides steep Ca²⁺ concentration gradients across both the ER membrane and the plasma membrane. Controlled opening of Ca²⁺ permeable channels in either membrane then allows Ca²⁺ to flow rapidly down its electrochemical gradient, generating the increase in cytosolic Ca²⁺ concentration that ultimately regulates cellular activity.

The versatility of these Ca²⁺ signals derives largely from their complex spatial and temporal organisation. Different Ca²⁺ channels, by delivering their Ca²⁺ to different subcellular domains, can selectively regulate distinct Ca²⁺ sensitive processes. Cellular responses may also be tuned to respond to the frequency of the ►Ca²⁺ spikes from which these Ca²⁺ signals are often built. Information is thus encoded in both the spatial and temporal organisation of Ca²⁺ signals. Both aspects depend upon interplay between Ca²⁺ channels targeted precisely to specific subcellular locations.

Whether Ca²⁺ signals remain local, regulating only proteins closely associated with active ►Ca²⁺ channels, or become global and so potentially regulate Ca²⁺ sensors throughout the cell, depends upon both the intensity of the initiating stimulus and the extent to which the Ca²⁺ signal propagates regeneratively across the cell. Signals detected at the plasma membrane may either directly stimulate Ca²⁺ channels within the plasma membrane (e.g., voltage-gated Ca²⁺ channels, NMDA receptors) or, via intracellular signals, cause activation of intracellular Ca²⁺ channels. The latter include IP₃ receptors, their relatives the ►ryanodine receptors (RyR), and the less thoroughly understood receptors for NAADP (nicotinic acid adenine dinucleotide phosphate). Each of these channels can contribute to initiation of Ca²⁺ signals, but stimulation of IP₃R

and RyR by cytosolic Ca²⁺ endows these channels with an additional ability to regeneratively propagate intracellular Ca²⁺ signals by ►Ca²⁺ induced Ca²⁺ release (Fig. 1). [1]

IP₃Rs open only when they bind IP₃: their ability to initiate and propagate Ca²⁺ signals therefore depends upon formation of IP₃, catalysed by ►phospholipases C which hydrolyse phosphatidylinositol 4,5-bisphosphate to IP₃ and diacylglycerol. Many receptors, responding to many different stimuli, lead to activation of phospholipase C and so to release of Ca²⁺ from intracellular stores via IP₃R. The same receptors almost invariably also stimulate Ca²⁺ entry across the plasma membrane, an essential response if Ca²⁺ signals are to be sustained because IP₃R within the ER have access to only a limited source of Ca²⁺. The links between these receptors and Ca²⁺ entry, and the nature of the Ca²⁺ entry pathway itself are diverse, but a common accompaniment of IP₃-evoked Ca²⁺ release is the activation of ►store-operated Ca²⁺ entry channels. These channels are formed from oligomeric assemblies of ►Orai proteins, which are activated by ►STIM proteins within the ER when the Ca²⁺ content of the ER falls allowing Ca²⁺ to dissociate from the luminal ►EF-hand of the STIM protein. IP₃Rs, therefore, not only initiate Ca²⁺ signals in response to the many receptors that stimulate IP₃ formation, they can regeneratively propagate the signals across the cell (Fig. 1), and they also, by depleting the ER of Ca²⁺, stimulate the store-operated Ca²⁺ entry required for sustained Ca²⁺ signalling.

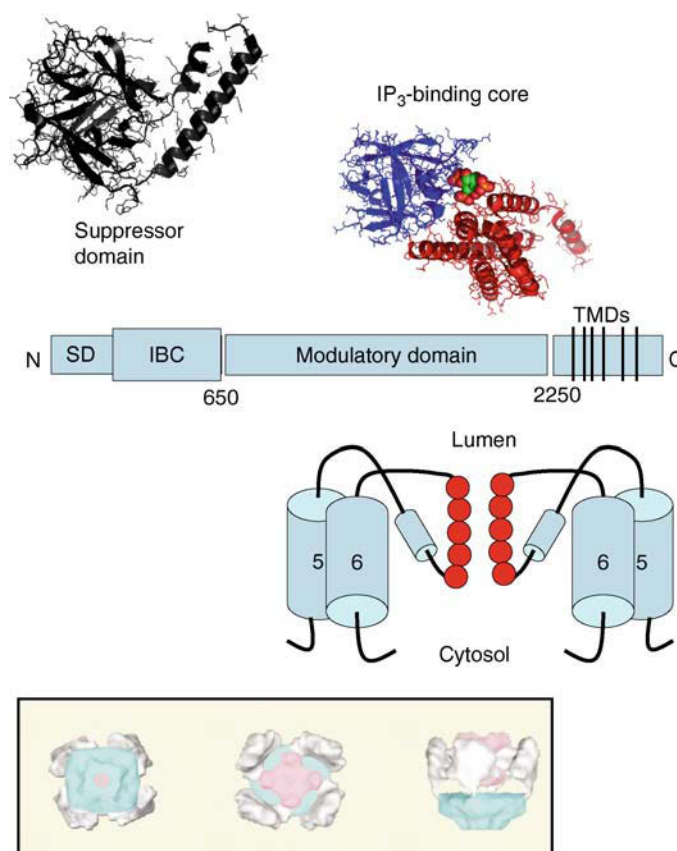


IP₃ Receptors. Figure 1 Interplay between Ca²⁺ channels. Ca²⁺ signals are initiated when an extracellular stimulus (red) directly opens a Ca²⁺ channel in the plasma membrane or indirectly, via a signalling pathway (green), opens an intracellular Ca²⁺ channel. Ca²⁺ signals may then be propagated across the cell by Ca²⁺-induced Ca²⁺ release mediated by IP₃R or RyR.

Structure of IP₃ Receptors

IP₃R are expressed in almost all animal cells, from *Caenorhabditis elegans* to man. They are found predominantly, though not exclusively, within the ER membrane. In vertebrates, three genes (and their splice variants) encode three distinct, but closely related, subunits of the IP₃R (IP₃R1–3) each comprising a large protein of about 2700 residues (~300 kDa). Functional IP₃R are tetramers: homotetramers in those species (e.g., insects and *C. elegans*) with only a single IP₃R subtype, but homo- or heterotetramers in vertebrates [2]. The essential properties of all IP₃R are broadly similar, but the subtypes differ in their distributions and in the finer details of their regulation. Here we concentrate on the key shared features, focussing primarily on IP₃R1, the most thoroughly studied subtype.

Each IP₃R subunit can be structurally divided into three regions: a single IP₃-binding site lying towards the N-terminal, a large central modulatory region, and six transmembrane domains (TMD) lying close to the C-terminus (Fig. 2). Residues 224–578 form the IP₃-binding core (IBC) and its crystal structure in complex with IP₃ shows IP₃ cradled at the corner of the L-shaped



IP₃ Receptors. Figure 2 Key structural features of IP₃ receptors. The key domains are shown in the central block. The upper structures show the suppressor domain (PDB accession code, 1XZZ) and the IBC (1N4K) with its (red) and β (blue) domains. A proposed structure for the pore region is shown below, with the selectivity filter shown in red; only two of the four subunits are shown. The lowest panel shows reconstructed 3D structures of IP₃R1 viewed (left to right) from ER lumen, the cytosol and in cross-section across the ER membrane (reproduced with permission from [4]).

structure formed by the α and β domains of the protein (Fig. 2) [3]. This binding cleft is lined by several key arginine and lysine residues that coordinate the three phosphoryl groups of IP₃. The 4- and 5-phosphates of IP₃ are essential for activity and while their major contacts are with the β and α domains, respectively, each makes some contacts with both domains. These interactions are consistent with a notion that activation of the IP₃R may be initiated by IP₃ drawing the α and β domains together into a more rigid structure. The 1-phosphate, by contrast, improves affinity, but it is not essential, and it makes far fewer contacts with the IBC. The N-terminal of the IP₃R comprises the so-called “suppressor domain” and its structure has also been resolved (Fig. 2) [4]. The suppressor domain is likely to be involved in coupling IP₃ binding to channel gating, but we do not yet know the relative dispositions of the IBC and suppressor domain in the native IP₃R structure. Towards the C-terminal of each subunit, there are

thought to be six helical TMDs; these target IP₃R to the ER, contribute to oligomerization, and form the cation-selective pore. The pore is formed by the last pair of TMD (TMD5–6) and the intervening luminal loop from each subunit. Its structure is thought to be similar to the pore of K⁺ channels, with the two TMD and a pore helix holding a relatively conserved selectivity filter (Fig. 2). Negatively charged residues flanking the luminal loop may contribute to the modest selectivity of the IP₃R pore for Ca²⁺ over monovalent cations (~sevenfold selective) by concentrating Ca²⁺ around the pore. A large cytoplasmic region (~1700 residues) separates the IP₃-binding domain from the TMDs. This region contains many (though certainly not all) sites through which IP₃R are modulated; it is often described as the “modulatory domain” [2]. It includes Ca²⁺-binding sites, sites to which ATP bind, sites for phosphorylation by several protein kinases, and sequences that mediate interaction with proteins like

calmodulin. Nothing is known of the structure of the modulatory domain.

At present only low resolution ($>30\text{\AA}$) structures, all derived from single particle analysis of images from electron microscopy, are available for the entire IP_3R . These structures differ in their details, but all show a roughly square structure with fourfold symmetry and lateral dimensions of about 20 nm (Fig. 2).

Regulation of IP_3 Receptors

IP_3 is the essential regulator of IP_3R : the pore opens only when the IP_3R has IP_3 bound. But IP_3 alone does not stimulate opening, rather it tunes the sensitivity of the IP_3R to Ca^{2+} by causing a stimulatory Ca^{2+} -binding site to become accessible and an inhibitory site to be occluded. Binding of Ca^{2+} to the stimulatory site then causes channel opening [5]. This interplay between IP_3 binding and Ca^{2+} regulation leads to biphasic effects of cytosolic Ca^{2+} on steady-state responses to IP_3 : modest increases in cytosolic Ca^{2+} promote channel opening, while more substantial increases are inhibitory. This pattern of regulation plays an essential part in the regenerative behaviour of IP_3R (Fig. 1). The identities of the essential Ca^{2+} -binding sites remain unclear. The stimulatory site is likely to reside on the IP_3R itself; while inhibition may be mediated by an accessory protein: **calmodulin** is a potential, albeit controversial, candidate.

IP_3 and Ca^{2+} are the essential regulators of IP_3R gating, but many additional signals and accessory proteins modulate their responses. Phosphorylation by a host of protein kinases, ATP, redox state, calmodulin, cytochrome c, and many additional proteins have all been shown to modulate IP_3R behaviour. IP_3Rs thus serve to integrate many different signals. These signals then tune the sensitivity of the IP_3R to IP_3 and Ca^{2+} , and so allow the IP_3R effectively to process information coming from many different signalling pathways before returning the output to the cytosol as a Ca^{2+} signal. IP_3 receptors are signal integrators.

How Does IP_3 Binding Lead to Opening of the Pore?

At present we can only speculate on the mechanisms that link IP_3 binding to the IBC to opening of the pore. IP_3 binding causes a large conformational change to the N-terminal region, perhaps initiated by a closure of the gap between the α and β domains of the IBC as IP_3 binds and cross-links the two domains. But how does IP_3 binding cause exposure of the essential stimulatory Ca^{2+} -binding site? A speculative possibility is that a cluster of acidic residues lying close to the hinge around which IP_3 is proposed to pull the α and β domains together might be re-arranged by that movement into a functional Ca^{2+} -binding site. The suppressor domain is essential for IP_3 to cause channel gating and may communicate directly with the pore region. We further

speculate that IP_3 and Ca^{2+} binding to the IBC free it from its interaction with the suppressor domain allowing it to then signal onwards to the pore, possibly by interacting directly with the cytosolic loop linking TMD4 and 5. Movement of that loop might then, in analogy with K^+ channels, physically gate the channel.

Pharmacological Intervention

No ligands of IP_3R have yet found clinical application. Most agonists of IP_3R are either modifications of IP_3 or derived from adenophostin A. The only known antagonists of IP_3R (e.g., heparin, 2-APB) are very poorly selective.

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IPC

Ischemic Preconditioning.

IPSP (Inhibitory Postsynaptic Potential)

An inhibitory postsynaptic potential is a local hyperpolarizing potential at a postsynaptic membrane, which is elicited by the release of an inhibitory neurotransmitter via an inhibitory postsynaptic current.

- Synaptic Transmission
- GABAergic System

IRAG

IP₃ receptor associated cGMP kinase substrate of 130 kDa that is present in all smooth muscles and platelets. It's phosphorylation decreases calcium release from intracellular IP₃-sensitive stores.

► Smooth Muscle Tone Regulation

IRAK Family

IL-1 receptor associated kinase family-A with Toll-like receptor signalling. There are four members in this group to date: IRAK-1,2,4 and M. They can phosphorylate among themselves, as well with other proteins involved in signalling such as ►TRAF-6.

► Toll-like Receptors

Iron Chelator

A molecule that binds iron through coordinating moieties (e.g., carboxylates or amines). They are used to inhibit iron-catalyzed free radical reactions or to treat iron overload conditions. Desferrioxamine and deferiprone are two widely used iron chelators.

Irreversible Antagonists

According to Michaelis–Menten kinetics, ligands have affinity for receptors determined by their rate of offset from the binding domain divided by their rate of onset to the binding domain. Reversible ligands occupy different proportions of receptor sites according to this ratio and the concentration present in the receptor compartment. Irreversible ligands have negligible rates of offset (i.e., once the ligand binds to the receptor it essentially stays there) therefore receptor occupancy does not achieve a steady-state but rather increases with increasing time of exposure of the receptors to the ligand. Thus, once a receptor is occupied by an

irreversible antagonist, it remains inactivated throughout the course of the experiment.

► Affinity

► Drug–receptor Interaction

Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome (IBS) is an exceedingly common condition in all societies, characterized by abdominal discomfort or pain in association with altered bowel habit or incomplete stool evacuation, bloating and constipation or diarrhoea. easily go undetected and do not show up with common tests such as blood tests or x-rays. The estimated prevalence in the community is about 10%. Irritable bowel syndrome and its variants, collectively called functional gastrointestinal disorders, constitute 40–50% of all the patients seen by gastroenterologists in Western countries.

► Serotonergic System

Isaacs' Syndrome

Isaacs' syndrome (an acquired neuromyotonia) is caused by autoantibodies directed against 4-aminopyridine or α -dendrotoxin-sensitive K⁺ channels (Kv1.1 and Kv1.6) in motor and sensory neurons. The syndromes include muscle twitching during rest, cramps during muscle contraction, impaired muscle relaxation, and muscle weakness due to hyperexcitability of peripheral motor nerves.

► K⁺ Channels

Ischemic Preconditioning (IPC)

IPC is the endogenous cellular protective mechanism in the heart by which brief periods of ischemia induce protection against infarction due to subsequent longer periods of ischemia.

► ATP-dependent K⁺ Channels

Ischemic Stroke

An episode of acute regional ischemia in the brain leading to neuronal death. It is usually caused by thrombi or emboli from atherosclerotic plaques.

► Atherosclerosis

Isopeptide Bond

An amide bond formed between a carboxyl group of one amino acid and an amino group of another, where either group occupies a position other than α .

Isoprenoid

Isoprenoids are intermediates and products of the biosynthetic pathway that starts with mevalonate and ends with cholesterol and other sterols.

► Lipid Modifications

Isoprenylation

► Lipid Modifications

ITAM

Immunoreceptor based activation motif. The classical ITAM motif comprises the consensus sequence YxxI/Lx(6–12)YxxI/L (where Y stands for tyrosine, I stands for isoleucine, L stands for leucine, and x can be any amino acid). Kinases containing tandem SH2 domains, as for example ZAP-70 or SYK, recognize the phosphorylated ITAMs, thereby initiating downstream signaling events.

► T Cell Receptors

Itch

(Itchy homolog E3 ubiquitin protein ligase) As revealed by cDNA cloning, the *itch* gene encodes 854 amino acids with a molecular weight of 113 kDa. As a regulatory E3 ubiquitin ligase, itch is composed of an N-terminal protein kinase, C-related C2 domain, four WW protein–protein interaction domains, and a C-terminal HECT ubiquitin ligase domain, and is a member of the HECT domain-containing E3 Ub protein ligases.

► Ubiquitin/Proteasome

► Transforming Growth Factor-Beta

JAK-STAT Pathway

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Definition

The *Janus kinase-signal transducer and activator of transcription* (JAK-STAT) signaling pathway is activated in response to a large number of ►cytokines, hormones, and growth factors. As their name implies, the ►STAT proteins exhibit the dual function of transducing signals from the cell surface into the nucleus as well as activating transcription of target genes, thus converting extracellular stimuli to a wide range of appropriate cellular responses [1, 2]. STATs have been identified as important regulators of a multitude of cellular processes, such as immune response, antiviral protection, and proliferation.

Basic Characteristics

The JAK-STAT pathway is widely used by members of the cytokine receptor superfamily. Upon ligand binding and oligomerization of the cognate receptor chains, the receptor-associated ►JAKs themselves become tyrosine phosphorylated and consecutively phosphorylate critical tyrosine residues on the cytoplasmic domain of the receptors, thereby generating docking sites for STAT proteins and other intracellular signaling molecules. The STATs are recruited to specific phosphotyrosine-containing motifs located in the cytoplasmic part of the receptor via their Src-homology-2 (SH2) domain and in turn are phosphorylated by activated JAKs at a single tyrosine residue in their C-terminus. Tyrosine phosphorylation induces conformational changes in the dimeric STAT molecules by virtue of reciprocal SH2–phospho-tyrosine interactions. The phosphorylated STATs are capable of binding to nonameric palindromes with relaxed sequence specificity in the promoter regions of cytokine-inducible genes, termed GAS (The gamma activated site is a

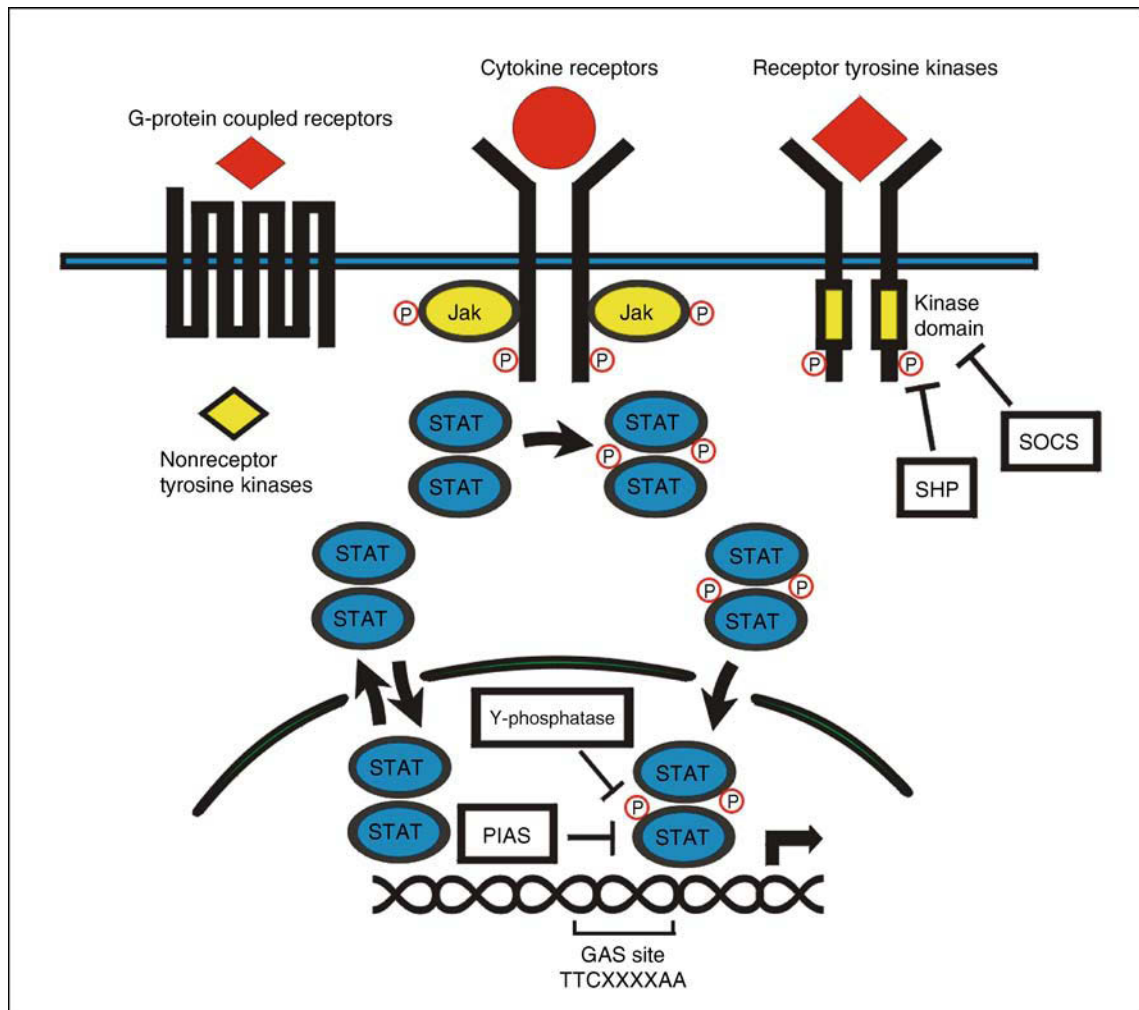
STAT binding site in the promoter region of cytokine-responsive genes. It is nonameric palindrome with relaxed sequence specificity.) sites, to regulate gene expression. ►Tyrosine phosphatases located in the nucleus then dephosphorylate STAT molecules (Fig. 1).

Physiological Functions of JAKs

The mammalian JAK family of protein tyrosine kinases consists of four members (JAK1, JAK2, JAK3, and TYK2), which are characterized by the possession of a kinase and an adjacent pseudokinase domain. JAKs have a molecular mass of approximately 130 kDa and are composed of seven JAK homology domains. The essential role of JAKs in mediating signal transduction via members of the cytokine receptor superfamily became apparent from studies of knockout mice. Targeted disruption of the mouse JAK1 gene results in perinatal lethality, obviously caused by defective neural function, and altered lymphoid development. JAK2-deficient mice exhibit an embryonic lethal phenotype caused by a block in definite erythropoiesis but show intact lymphoid development, demonstrating the obligatory and nonredundant roles of JAKs in cytokine-induced biological responses. Mutant mice lacking JAK3 are viable but display severe defects in both cellular and humoral immune responses with profound reduction in mature B and T cells, resembling the clinical symptoms of patients suffering from an autosomal-recessive form of severe combined immunodeficiency in which inactivating mutations in the JAK3 gene have been identified.

Roles of STAT Proteins in Cytokine-Inducible and Constitutive Gene Induction

In mammalia, seven different members encoded by distinct genes have been identified, all of which are activated by a distinct set of cytokines. Diversity in signaling is provided by variants of STAT proteins derived from either alternative splicing of RNA transcripts or proteolytic processing (e.g., STATs 1, 3, 4, and 5) and the ability of certain STATs to form both homodimers and heterodimers with each other. In response to ►interferon- γ monomeric STAT1 dimerizes, while upon interferon- α stimulation a heterotrimeric complex consisting of STAT1 and STAT2 with associated



JAK-STAT Pathway. Figure 1 Activating and inhibitory mechanisms of the JAK-STAT pathway.

p48 is formed, known as the ISGF3 ▶ **transcription factor**. Major structural features in STAT proteins are the N-terminal region involved in cooperative DNA binding of multiple STAT dimers, the central DNA-binding domain, the dimerization region containing the SH2 domain and the site of tyrosine phosphorylation, and the C-terminal transcriptional transactivation domain. Phosphorylation of a critical serine residue within the transactivation domain is necessary for maximal transcriptional activity of some STAT family members.

STAT1 knockout mice exhibit selective signaling defects in their response to interferon, including an impaired expression of MHC class II, complement protein C3, the MHC class II transactivating protein CIITA, interferon regulatory factor-1, and guanylate-binding protein [3]. STAT3 activated through binding of IL-6, leptin, EGF, PDGF, LIF, or other ligands to their cognate receptors appears to have important roles in preventing apoptosis and promoting proliferative processes. A deficiency in STAT3 causes embryonic

lethality in mice, indicating the essential role of STAT3 in growth regulation, embryonic development, and organogenesis. STAT4 is activated in T cells in response to IL-12 and stimulates the development of T_H1 cells. The two ubiquitously expressed STAT5 proteins (STAT5a and 5b) are encoded by distinct genes and share more than 90% sequence identity. They are activated by many growth stimulatory cytokines, including interleukins, GM-CSF, GH, prolactin, EGF, as well as erythropoietin, and exert critical roles in antiapoptosis and proliferation. STAT6 functions in response to IL-4 and IL-13 signaling to induce T_H2 cell development, CD23 and MHC class II expression, immunoglobulin class switching, and B- and T-cell proliferation.

Originally discovered as DNA-binding proteins that mediate interferon signaling, recent data demonstrated that STAT1 can also exert constitutive functions in the nucleus, which do not require STAT activation with tyrosine phosphorylation. Cells lacking STAT1 are

resistant to apoptotic cell death induced by tumor necrosis factor due to an inefficient expression of caspase genes, while reintroduction of STAT1 in these cells restores protease expression and sensitivity to apoptosis. For the transcription of certain target genes a phosphorylation of the critical tyrosine residue 701 is not necessary, suggesting that unphosphorylated STAT1 can also bind to DNA. Recent data indicate that unphosphorylated STAT1 can either positively or negatively regulate the constitutive expression of a wide range of different genes. It was shown that tyrosine phosphorylated and unphosphorylated STAT1 molecules shuttle between the cytoplasm and the nucleus via independent pathways to distinct sets of target genes.

Besides the cytokine receptors that lack intrinsic kinase activity but have associated JAK kinases, STAT proteins can be activated by a variety of G-protein coupled receptors and growth factor receptors with intrinsic tyrosine kinase activity (for example EGF, PDGF, CSF-1, and angiotensin receptor). Increasing evidence suggests a critical role for STAT family members in oncogenesis and aberrant cell proliferation. Constitutively activated STATs have been found in many transformed cell lines and a wide variety of human tumor entities. Numerous non-receptor tyrosine kinases and viral oncoproteins, such as v-Src, v-Abl, v-Sis, and v-Eyk, have been identified to induce DNA-binding activity of STAT proteins.

Mechanisms negatively regulating the JAK-STAT pathway have been identified. Besides the dominant-negative effects of naturally occurring STAT variants lacking the transactivation domain, ►PIAS proteins (protein inhibitor of activated STAT) have been shown to interact with STATs and to suppress their DNA-binding activity. The ►SOCS proteins (suppressor of cytokine signaling), also named as CIS (cytokine inducible src homology 2-domain containing protein), JAB (JAK-binding protein) or SSI (STAT-induced STAT inhibitor), are induced by cytokine signaling and act in a negative feedback loop to inhibit JAK kinase activity. Another important negative regulatory mechanism involves the recruitment of tyrosine phosphatases containing tandem SH2 domains (SHP-1 and SHP-2) to the intracytoplasmic portion of receptor complexes, where they dephosphorylate and thus inhibit JAK activity.

Drugs

Components of the JAK-STAT signaling pathway represent novel targets for pharmacological interventions [4]. Recently, a specific and orally active JAK3 antagonist was identified from screening of a chemical library for inhibitors of in vitro JAK3 kinase activity. The most effective compound, CP-690,550, was shown

to significantly suppress immune reactions and prevent allograft rejection in animal organ transplantation [5]. The JAK3 inhibitor significantly prolonged survival in a murine model of heart transplantation and in cynomolgus monkeys receiving kidney transplants. Moreover, typical side effects associated with established immunosuppressive therapies were not reported, suggesting a potential role for CP-690,550 or derivatives thereof in the prevention and treatment of transplant rejection and other clinical conditions of desirable immunosuppression.

Other interesting targets for pharmacological therapies include the STAT proteins. Drugs specifically blocking dimeric STAT molecules are of promising value in the treatment of cancer, because of the high levels of STAT activation in tumor cells. Structural targets for the development of novel therapeutics within the STAT molecule include the SH2 domain responsible for dimerization and recruitment to the receptor, the DNA-binding domain as well as the recently discovered nuclear import signal for activated STAT1.

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JNKs

c-jun N-terminal kinases. Group of three kinases (JNK1, JNK2, JNK3) which belongs to the family of mitogen-activated protein (MAP) kinases and phosphorylate c-Jun on Ser63 and Ser73. JNK1 and JNK2 are widely expressed, whereas JNK3 is primarily found in the nervous system and the testes. JNKs are activated by various stress signals like cytokines, ultra-violet irradiation or osmotic shock.

►MAP Kinase Cascades

K_{ATP} Channel

► ATP-dependent K⁺ Channel

K_{NDP} Channel

► NDP-dependent K⁺ Channels

K⁺ Channel Openers

Typical KCO members are diazoxide, pinacidil, cromakalim, and nicorandil. KCOs activate K_{ATP} channels by binding to SUR subunits. Diazoxide and nicorandil are clinically used in treatment of PHHI and angina pectoris, respectively.

► ATP-dependent K⁺ Channels

K Channels

K Channels belong to a class of membrane proteins that form highly K-selective pores in membranes. All known K Channels are composed of several (usually four) pore forming alpha subunits and auxiliary beta subunits. K Channels play an essential role in cellular excitability, being involved in repolarization of Action Potentials and setting the cell resting potential as well as contributing to potassium homeostasis.

► Potassium Channels

K_D

The dissociation constant for the drug–receptor complex. It is a measurement for the affinity of a drug to a receptor. The lower the value, the higher the affinity.

K⁺ Homeostasis

Potassium homeostasis refers to the maintaining and regulating of a relatively stable and mostly internal (intracellular) potassium balance (concentration), although more generally it refers to the maintenance of potassium balance in any compartment (e.g. in the blood).

► K⁺ Channels

k_{off}

K_{off}, or the dissociation constant of a drug, refers to the rate at which the drug-receptor complex dissociates into separate drug and receptor units.

Kainate Receptor

Kainate receptors are a subtype of ionotropic glutamate receptors that are permeable to Na⁺, K⁺ and Ca²⁺ ions.

► Ionotropic Glutamate Receptors

Kallidin, Lysyl-Bradykinin

► Kinins

Kallikrein

► Kinins

KCNQ-Channels

KCNQ-channels are assembled from KCNQ α -subunits. Mutations in the KCNQ1 gene are associated with heart arrhythmia and deafness (LQT1 syndrome, Romano–Ward Syndrome, Jervell–Lange Nielsen Syndrome). Mutations in KCNQ2 and KCNQ3 genes are associated with a benign form of juvenile epilepsy (BFNC). Mutations in the KCNQ4 gene are associated with deafness.

- Antiepileptic Drugs
- Voltage-gated K^+ Channels
- K^+ Channels

KCOs

► K^+ Channel Openers

KDEL Receptor

Interaction of the KDEL-receptor with soluble luminal proteins bearing the tetrapeptide KDEL at their carboxy-terminus retrieves these proteins back from the Golgi to the endoplasmic reticulum.

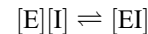
► Intracellular Transport

KELL Blood Group Antigen

KELL blood group antigen is a plasma membrane protein isolated from red cells homologous to zinc-binding glycoproteins with neutral endopeptidase activity.

Ki Values

The K_i value is the dissociation constant of an enzyme-inhibitor complex. If $[E]$ and $[I]$ are the concentrations of enzyme and its inhibitor and $[EI]$ is the concentration of the enzyme-inhibitor complex, there is an equilibrium of complex formation and detachment as follows:



Under these circumstances, K_i can be defined as:

$$k_i = \frac{[E][I]}{[EI]}$$

Kinase

A kinase is an enzyme that catalyzes the transfer of the terminal phosphate of a nucleotide to suitable substrates. Protein and lipid kinases play important roles in signaling. The kinase domain is a two lobed structure with an ATP binding site and a substrate-binding site. Protein kinases can be tyrosine kinases (e.g. receptor tyrosine kinases (RTKs)), serine/threonine kinases (e.g. protein kinase C – PKC) or dual specificity kinases (e.g. MEK). Kinases that can phosphorylate histidine and arginine residues have been identified in lower organisms.

- Tyrosine Kinases
- Protein Kinase Inhibitors

Kinase Domain

► Kinase

Kinase Inhibitors

- Tyrosine Kinase Inhibitors
- Protein Kinase Inhibitors

Kinins

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Synonyms

Bradykinin; Kallidin (lysyl-bradykinin); desArg⁹-bradykinin; desArg¹⁰-kallidin (Lys⁰, desArg⁹-bradykinin); T-kinin (Ile-Ser-bradykinin)

Definition

Kinins are a group of peptide hormones of 8–11 residues that act locally as proinflammatory agents, often through the release of powerful downstream effectors such as nitric oxide and/or prostaglandins.

Basic Characteristics

Liberation of Kinins

Typically, kinins are locally produced at injured or inflamed tissues sites where they act through a combined endocrine/paracrine mode. In a first step, the high-molecular weight inactive kinin precursor proteins, the kininogens, are targeted to the inflamed site where the kinins are eventually proteolytically liberated from the kininogens by specific kininogenases, the kallikreins. In humans, two types of kininogens exist, termed high-molecular-weight (H-) kininogen (H-kininogen) and low-molecular-weight (L-) kininogen (Fig. 1.). Both types of kininogens are encoded by distinct mRNAs generated by alternative splicing from a single kininogen gene. A third type of kininogen, T-kininogen, appears to be unique for rats and is not found in humans or other mammals. Kininogens are predominantly expressed by hepatocytes and secreted into the human plasma via constitutive routes. Kidney and secretory glands are among the prominent extrahepatic sites of kininogen production.

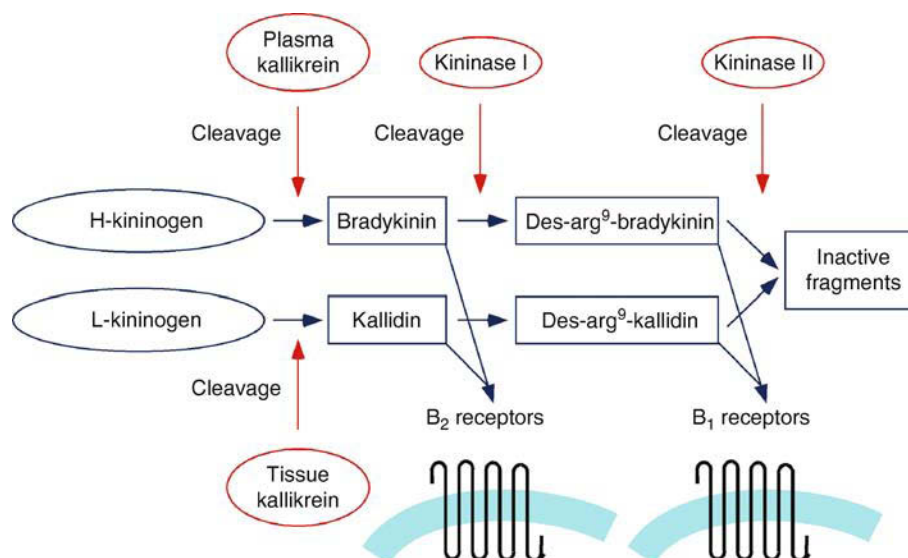
The most common kinin-liberating enzymes are kallikreins, a group of serine proteases that are found in glandular cells, ►neutrophils and biological fluids such as plasma and urine. In humans, two classes of kinin-liberating kallikreins exist i.e. tissue kallikrein and plasma kallikrein, that differ by their structural, immunological and functional characteristics [1]. In some mammalian species, tissue kallikrein preferentially utilizes L-kininogen as the substrate to produce kallidin, whereas plasma kallikrein acts on H-kininogen to generate bradykinin (Table 1). Kinins can also be released by common proteases such as trypsin, elastase or cathepsin D, however the biological significance of these kinin-liberating processes is unknown. Apart from human kininogenases, a large number of proteases derived from pathogenic microorganisms has been found to liberate kinins from kininogens.

Kinins are extremely short-lived peptide hormones (plasma half live <15 s) that are rapidly proteolytically converted and inactivated. For instance, bradykinin and kallidin are cleaved at their carboxy-terminal ends by carboxypeptidases of the N and M type, collectively referred to as kininases type I, to form desArg⁹-bradykinin and desArg¹⁰-kallidin, respectively (Table 1). Because bradykinin and kallidin act on B₂ receptors, and desArg⁹-bradykinin and desArg¹⁰-kallidin bind exclusively to B₁ receptors, the proteolytic conversion of the kinins “switches” the relative binding affinity for the two kinin receptors, B₁ and B₂ receptor, respectively (Fig. 1.) Both types of agonists are rapidly degraded and inactivated by the action of the dipeptidylpeptidase angiotensin-converting enzyme, also known as kininase type II, and neutral endopeptidase which inactivate kinins by trimming the kinin peptides at their carboxy-terminal ends (Table 1).

In humans as well as in other but not all mammalian species, kininogens are modified by posttranslational hydroxylation of a single proline residue of their kinin sequence, i.e. position 3 in bradykinin or position 4 in kallidin. Hydroxylation appears not to affect the specificity, affinity or intrinsic efficacy of the kinins.

Kinins in Biological Fluids

The quantification of kinins in human tissues or body fluids has been limited due to the inherent difficulties in accurately measuring the concentration of ephemeral peptides. Today HPLC-based and RIA/capture-EIA measurements are established to determine kinins in human plasma, liquor or urine. Serine protease inhibitors need to be added to prevent rapid degradation of the kinins *in vitro* during sample preparation. Kinins and their degradation products have been studied in various biological milieus such as plasma/serum, urine, joint fluids, kidney, lung and skeletal muscle [2]. Under normal conditions, the concentration of kinins in these compartments is extremely low; for



Kinins. Figure 1 Scheme of kinin liberation, turnover and action.

Kinins. Table 1 Enzymes involved in the generation and degradation of kinins

Substrate	Processing enzyme	Major Product	Kinin receptor
H-kininogen	Plasma kallikrein	bradykinin (RPPGFSPFR)	B ₂ receptor
	Tissue kallikrein	kallidin	
L-kininogen	Tissue kallikrein	kallidin (KRPPGFSPFR)	B ₂ receptor
	Plasma kallikrein	bradykinin	
bradykinin	Carboxypeptidase N	desArg ⁹ -bradykinin (RPPGFSPF)	B ₁ receptor
	Carboxypeptidase M	bradykinin 1–7 (RPPGFSP)	Inactive
	Neutral endopeptidase (NEP)	bradykinin 1–7 (RPPGFSP)	Inactive
	Angiotensin converting enzyme (ACE)	1–5 (RPPGF)	Inactive
kallidin	Carboxypeptidase N	desArg ¹⁰ -kallidin (KRPPGFSPF)	B ₁ receptor
	Carboxypeptidase M	kallidin 1–8 (KRPPGFSP)	Inactive
	Neutral endopeptidase (NEP)	kallidin 1–8 (KRPPGFSP)	Inactive
	Angiotensin converting enzyme (ACE)	1–6 (KRPPGF)	Inactive
desArg ⁹ -bradykinin (RPPGFSPF)	Neutral endopeptidase (NEP)	bradykinin 1–7 (RPPGFS), 1–4 (RPPG)	Inactive
	Angiotensin converting enzyme (ACE)	bradykinin 1–5 (RPPGF)	Inactive
desArg ¹⁰ -kallidin (KRPPGFSPF)	Neutral endopeptidase (NEP)	kallidin 1–5 (KRPPG)	Inactive
	Angiotensin converting enzyme (ACE)	kallidin 1–6 (KRPPGF)	Inactive

instance kinin levels in human plasma are in the femtomolar and lower picomolar range. Plasma concentration of kininogens is in the micromolar range, i.e. the ratio of effector to precursor concentrations is maintained at an extremely low level of 10^{-6} to 10^{-9} , and therefore kinin release must be extremely tightly controlled. Kinin levels, however, can considerably rise in patients prone to diseases such as hereditary angioedema. In more severe complications such as ►sepsis and ►septic shock, kinin levels correlate with the severity of the disease state.

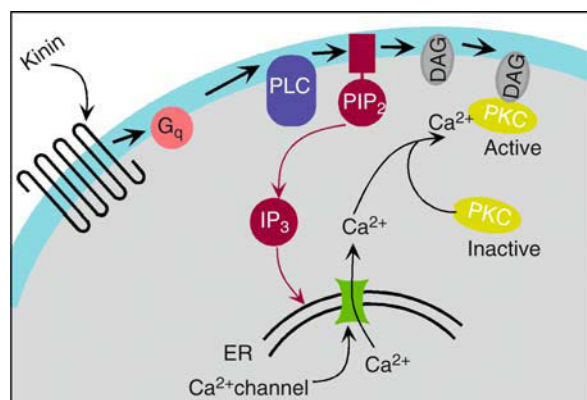
Receptors

The pathophysiological kinin functions are mediated by their interaction with specific receptors. In humans, two types of kinin receptor have been identified, namely B₁ and B₂ receptors [1]. The two receptors are coded by two genes on closely opposed loci of human chromosome 14q32 that likely arose from a common progenitor, though their sequence identity is limited. The two proteins belong to the large family of G protein-coupled receptors characterized by seven transmembrane-spanning helices. Unlike B₂ receptors that are constitutively expressed

in many cell types and tissues, the expression levels of B_1 receptors are very low under non-stimulated conditions. However, inflammatory or noxious stimuli boost the biosynthesis of the B_1 receptor. Several ►cytokines such as interleukin IL-1 β , as well as ►growth factors and bacterial ►lipopolysaccharides can up-regulate the transcription of the B_1 gene. B_1 and B_2 receptors are predominantly coupled to the ►pertussis toxin-insensitive G_q type of G protein leading to phospholipase C activation, mobilization of intracellular calcium by inositol-1,4,5-trisphosphate (IP₃) and activation of ►protein kinase C (Fig. 2.) Kinin receptors are vigorous stimulators of the biosynthesis of potent downstream effectors such as ►prostaglandins and ►leukotrienes due to ►phospholipase A₂ activation, and of ►nitric oxide via stimulation of the endothelial isoform of ►nitric oxide synthase (eNOS).

Kinin Receptor-Deficient Mice

Mice that are homozygous for a disrupted B_1 or B_2 receptor gene are healthy, fertile and normotensive. In B_1 -deficient mice, bacterial lipopolysaccharide-induced ►hypotension is diminished and the recruitment of polymorphonuclear leukocytes to the sites of tissue injury is impaired, and the animals show signs of ►hypoalgesia. Deletion of the B_2 gene in mice leads to salt-sensitive hypertension and altered ►nociception.



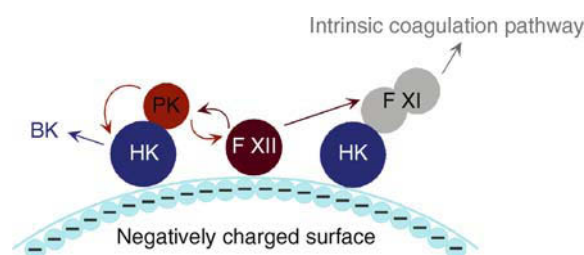
Kinins. Figure 2 Intracellular signaling cascades triggered by kinins. Following binding of kinins to the receptor the associated heterotrimeric G protein complex dissociates. The α subunit stimulates phospholipase C (PLC) which in turn catalyses the breakdown of phosphatidylinositol-4,5-bisphosphate (PIP₂) into 1,2-diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP₃). IP₃ reacts with Ca²⁺ channels in the endoplasmic reticulum (ER) releasing Ca²⁺ into the cytosol. The increase in intracellular Ca²⁺ levels activates protein kinase C (PKC), which translocates to the plasma membrane, anchoring to DAG and phosphatidylserine.

B_2 knockout embryos subjected to salt stress *in utero* show suppressed renin expression and an abnormal kidney phenotype and develop early postnatal hypertension. Consistently, although basal bradykinin formation is defective tissue kallikrein-null mice have normal blood pressure however suffer from cardiovascular abnormalities. However suggesting a function of kinin signaling during development.

Biological Functions and Clinical Implications of the Cardiovascular System

Kinins are implicated in many physiological and pathological processes including the induction of pain and hypotension, contraction of smooth muscles, regulation of local blood flow, stimulation of electrolyte fluxes, activation of sensory neurons and increase of vascular permeability. Many of these effects are triggered at least in part via the major downstream effectors ►prostaglandins, leukotrienes, the small GTPase Cdc42 and ►nitric oxide.

In rare cases of a systemic release, kinins have the potential to cause severe hypotension. Uncontrolled activation of the contact system (Fig. 3) is thought to trigger a massive formation of kinins under certain pathological conditions [3]. For instance, this situation is seen in patients with underlying diseases such as systemic inflammatory response syndrome (SIRS) due to sepsis or trauma. SIRS progression is accompanied by depletion of contact system factors and low levels of H-kininogen and plasma kallikrein are indicative of a



Kinins. Figure 3 Activation of the contact system on negatively charged surfaces. Upon perturbation or damage, endothelial cells change their properties from a non-thrombogenic to a thrombogenic state, thereby initiating pro-coagulative and pro-inflammatory cascades. The contact system contributing to this conversion comprises four factors, i.e. three inactive proteinases, factor XII (F XII, Hageman factor), factor XI (F XI), plasma prekallikrein (PK), and a single non-enzymatic co-factor, H-kininogen (HK). Under physiological conditions, these proteinases circulate as zymogens. The contact system associates with glycosaminoglycans of cell surface proteoglycans exposed by many cell types of the cardiovascular system, such as platelets, leucocytes and endothelial cells.

fatal outcome, as often found in sepsis [4]. Other diseases associated with a massive, often systemic release of kinins are hereditary angioedema and pancreatitis. Kinins are also generated during allergic and non-allergic rhinitis and ▶asthma. Notably, kinins are important endogenous mediators exerting acute protective effects in the ischemic myocardium via ▶nitric oxide-dependent mechanisms. On the other hand, stable synthetic kinin homologues, which efficiently open the endothelial barrier, are utilized in cancer therapy. These kinin receptor agonists facilitate penetration of chemotherapeutic drugs from the blood into adjacent tissues.

In injured arterial vessels reciprocal activation of FXII and plasma prekallikrein results in a local accumulation of active kallikrein which in turn cleaves its substrate H-kininogen and releases bradykinin (BK). Active FXII triggers the intrinsic pathway of coagulation via its substrate factor XI. Although FXII is activated by a variety of polyanions, including constituents of subendothelial matrix (glycosaminoglycans, collagens, sulfatides, and nucleosomes) and non-physiological materials (glass, ellagic acid, kaolin, silica) the mechanism(s) responsible for FXII activation *in vivo* are not completely understood. Induction of fibrin clot formation through contact activation-mediated activation of FXII is the basis of the activated partial thromboplastin time (aPTT) assay, a commonly used method for the global assessment of plasma coagulation in clinical settings. Deficiency of contact proteins H-kininogen, plasma kallikrein or FXII severely prolongs the aPTT but does not cause bleeding in patients. Although FXII-mediated fibrin formation plays no significant role for hemostasis, FXII-deficient mice are protected from arterial and venous thrombus formation and ischemia-reperfusion injury in models of ischemic stroke [5].

Drugs

Aprotinin (Trasylol®) was the first drug to be used in the clinic to prevent the formation of kinins. More recently this potent serine protease inhibitor has received much attention in cardiac surgical practice as a pharmacologic intervention to improve the hemostatic derangement associated with cardiopulmonary bypass. Interestingly, aprotinin isolated from bovine lung not only inhibits contact activation but also impinges on a number of interrelated pathways, thereby providing an antifibrinolytic effect, attenuating platelet dysfunction and down-regulating inflammatory responses. As a drug, aprotinin is also used to reduce blood loss and transfusion requirements in patients with a risk of hemorrhage, though the underlying molecular mechanisms responsible for the beneficial effects of aprotinin are still obscure. However, due to aprotinin's anaphylactic potential, the use of Trasylol has raised serious

concerns worldwide. Recently, a specific inhibitor of active plasmakallikrein (DX88®) was introduced for treatment of attacks of hereditary angioedema. Alternatively, a peptide based inhibitor of B₂ receptors (icatibant®) is used in clinical trials to prevent life threatening swellings hereditary angioedema and in severe scernings.

In addition to this inhibitor, a large number of specific B₁ and B₂ receptor antagonists has been developed. Some of them are orally available and are resistant to proteolytic degradation. Kinin antagonists have been tested in clinical trials and it appears that they have sepsis, effects on the survival in patients with severe systemic inflammatory response syndrome and sepsis and in patients suffering from severe traumatic brain injury. Kinin antagonists are drug candidates for diseases such hyperalgesia and angioedema, and the use of kinin agonists in the treatment of brain tumors is anticipated.

Angiotensin-converting enzyme (ACE) inhibitors represent a class of drugs that have proven anti-hypertensive and anti-proteinuric effects. They delay the progression of renal disease in conjunction with the ability to reduce systemic blood pressure. Furthermore they have been shown to reduce mortality and morbidity in myocardial infarction associated with chronic heart failure. The cardioprotective effect of ACE inhibitors is a combined result of the diminished conversion of angiotensin I and of the attenuated kinin breakdown leading to kinin accumulation in ischemic myocardia. Given their pleiotropic effects, ACE inhibitors may well use alternative mechanism(s) to exert their beneficial roles, e.g. through the resensitization of kinin receptors, however, the precise modes of action remain to be determined.

▶Inflammation

▶Nociception

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Kir Channels

Kir Channels are a class of potassium channels generated by tetrameric arrangement of one-pore/two-transmembrane helix (1P/2TM) protein subunits, often associated with additional beta subunits. Kir Channels serve to modulate cell excitability, being involved in repolarization of Action Potentials, setting the resting potential of the cell and contributing to potassium homeostasis.

- Inward Rectifier K⁺ Channels
- K⁺ Channels

Knockout Mice

Knockout mice are mice carrying a mutation leading to the disruption of a certain gene. Using a molecular genetic technique called “gene targeting,” a gene is rendered nonfunctional in totipotent embryonal stem cells in culture. These mutant cells are then used to generate mice carrying the mutation in the germ line, thus leading to the establishment of an animal colony with a loss of function of the desired gene.

- Transgenic Animal Models

Kringle Domains

Conserved sequences that fold into large loops stabilized by three disulfide linkages. The name Kringle comes from the Scandinavian pastry that these structures resemble. They can mediate certain protein–protein interactions.

- Growth Factors

K⁺-Sparing Diuretics

- Diuretics

Kvβ -Subunits

Kvβ -subunits are auxiliary subunits of Shaker-related Kv-channels, which belong to the Kv1 subfamily of voltage-gated potassium channels. Kvβ -subunits may function as chaperones in Kvα -subunit assembly and may modulate the gating properties of Kv-channels. In particular, some Kvβ -subunits may confer a rapid inactivation to otherwise non-inactivating Kv-channels.

- Voltage-gated K⁺ Channels

Kv-Channels

Kv-channel is an abbreviation of voltage-gated potassium channels. K stands for potassium and v for voltage.

- Voltage-gated K⁺ Channels

KvLQT1-Channels

The subunits of KvLQT1-channels were cloned by positional cloning from mutant human DNA obtained from patients suffering from a hereditary LQT1 (long QT) syndrome associated with heart arrhythmia and deafness. The corresponding gene is abbreviated as KCNQ1. KCN stands for K-channel, Q for the K-channel subfamily, and 1 indicates it as the first member of the KCNQ subfamily. Relatives of this channel (KCNQ2-KCNQ5) underlie the M-channels in the nervous system.

- M-Channels
- K⁺ Channels
- Voltage-gated K⁺ Channels
- K⁺ Channels

Kynurenine Pathway

The kynurenine pathway accounts for most of the non-protein tryptophan metabolism in most tissues. Several metabolites produced by this pathway have been shown

to be biologically active. Kynurenic acid produced via kynurenine has been shown to be an antagonist at N-methyl D-aspartate (NMDA), kainate and α -amino-5-methyl-3-hydroxy-4-isoxazole propionic acid (AMPA) receptors. It can block glutamate receptors in various species in distinguish subpopulations of kainate

receptors. 3-hydroxykynurenine can produce neuronal damage by generating radicals. Quinolinic acid, another metabolite produced by the kynurenine pathway, functions as an agonist at NMDA receptors.

► Ionotropic Glutamate Receptors

L-NAME

L-NAME (N-nitro-L-arginine methyl ester), like L-NMMA, is a structural analogue of L-arginine and competes with L-arginine for NO-synthase, which uses L-arginine as a substrate for the formation of NO. L-NMMA and L-NAME are very effective NO-synthesis inhibitors, both in vitro and in vivo.

►NO Synthase

β-Lactam Antibiotics

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Synonyms

Wall peptidoglycan inhibitors

Definition

►β-Lactam antibiotics are bicyclic or monocyclic azetidinone ring-containing compounds (Fig. 1). They kill bacteria by preventing the assembly of (4–3) peptidoglycans. These covalently closed net-like polymers form the matrix of the cell wall by which the bacteria can divide and multiply, despite their high internal osmotic pressure.

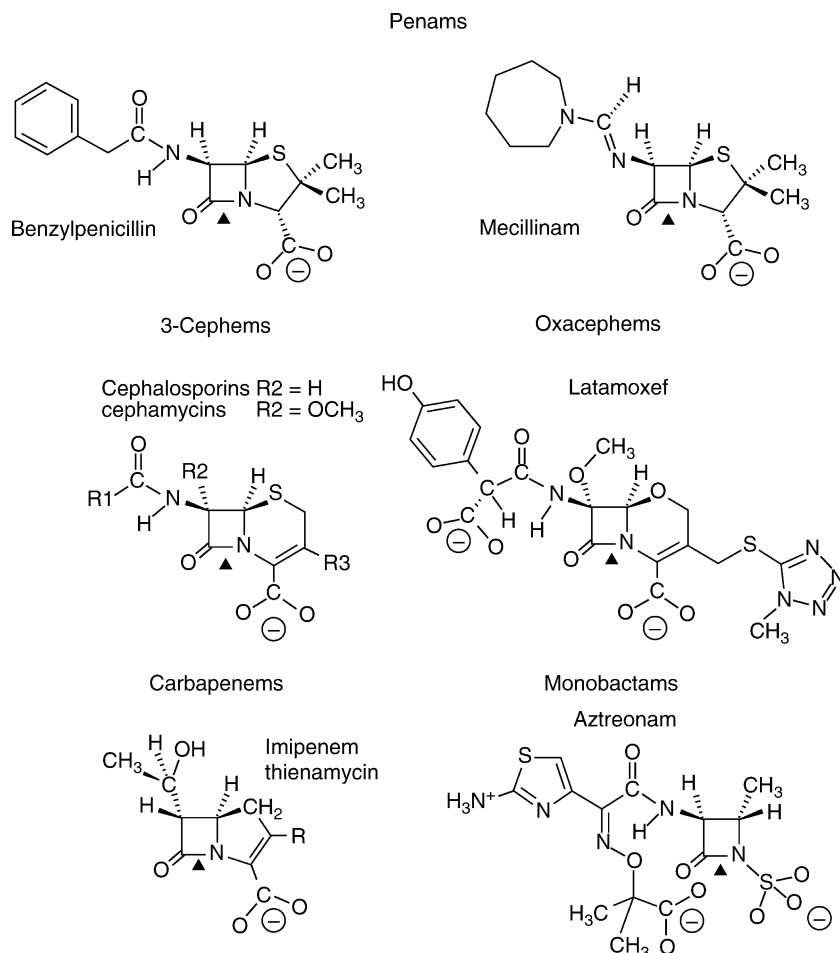
Mechanism of Action

►Peptidoglycans of the (4–3) and (3–3) types (Fig. 2) are comprised of glycan chains made of alternating β-1,4-linked *N*-acetylglucosamine and *N*-acetylmuramic acid residues [1]. The D-lactyl groups on carbon C₃ of the muramic acids are substituted by L-alanyl-γ-D-glutamyl-L-diaminoacyl-D-alanine stem tetrapeptides. In the (4–3) peptidoglycans, peptides borne by adjacent glycan chains are cross-linked through direct linkages or cross bridges (comprising one or several intervening

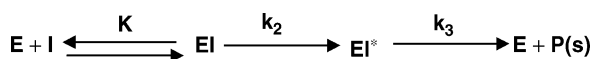
amino acid residues) that extend from the D-alanine residue at position 4 of a stem peptide to the ω-amino group at position 3 of another stem peptide. Lipid II (Fig. 3) is the immediate biosynthetic precursor. A disaccharide bearing an L-alanyl-γ-D-glutamyl-L-diaminoacyl-D-alanyl-D-alanine stem pentapeptide (the diamino acid residue of which can be either free, i.e., unsubstituted, or substituted by one or several amino acid residues, i.e., branched) is exposed on the outer face of the plasma membrane, linked to a C₅₅-undecaprenyl via a pyrophosphate. From this precursor, the formation of polymeric (4–3) peptidoglycans relies on glycosyl transferases ensuring glycan chain elongation and acyl transferases ensuring peptide cross linking. Acyl transferases of the SXXK superfamily (►SXXK Acyl Transferases, also called DD-transpeptidases, with X denoting a variable amino acid residue) are implicated in cross linking and they exhibit a specific bar code in the form of three motifs, SXXK, SXN (or analogue) and KTG (or analogue), occurring at equivalent places and roughly with the same spacing along the polypeptide chains [2]. As a result of the polypeptide folding, the motifs are brought close to each other at the immediate boundary of the catalytic centre between an all-α domain and an α/β domain. SXXK acyl transferases identify *N*-acyl-D-alanyl-D-alanine sequences as carbonyl donors, produce a *N*-acyl-D-alanyl moiety linked as an ester to the serine residue of the invariant SXXK motif and transfer the peptidyl moiety onto an amino group (transpeptidation) or a water molecule (carboxypeptidation). SXXK acyl transferases identify penicillin (used as a generic term for β-lactam antibiotics) as a suicide carbonyl donor. Because the serine-ester linked penicilloyl enzyme that SXXK acyl transferases produce is rather stable, they are immobilized, at least for a long time, in the form of penicillin-binding proteins, in short PBPs. The inactivation reaction can be written



where E-OH is the enzyme, with –OH the hydroxyl group of its active-site serine residue. Kinetically, the interaction is described by a 3-step model



β -Lactam Antibiotics. Figure 1 Bicyclic (penams, 3-cephems, oxacephems and carbapenems) and monocyclic (aztreonam) β -lactam antibiotics. Rupture of the scissile amide bond of the azetidinone ring (arrow) by the SXXK acyl transferases implicated in (4–3) peptidoglycan synthesis results in the formation of long-lived, serine-linked acyl ester derivatives. The inactivated enzymes behave as penicillin-binding proteins or PBPs.

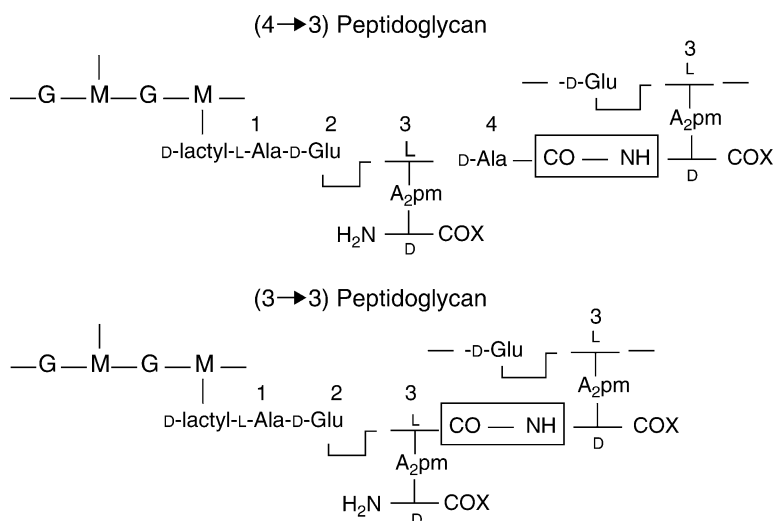


where I is the β -lactam, P(s) the product(s) resulting from the very slow decay of the EI* penicilloyl enzyme. K is the dissociation constant of the EI, a non-covalent complex and k_2 and k_3 first-order rate constants. In all cases, k_3 has been found to be very small and K is generally large so that the sensitivity of a PBP to a β -lactam is best characterized by the k_2/K ratio [3]. This ratio varies from $1,000,000 \text{ M}^{-1}\text{s}^{-1}$ for the most sensitive PBP to less than 1 for the most resistant.

Lethal Target Proteins

A constellation of genes code for PBPs of varying amino acid sequences and functionalities. PBPs occur as free-standing polypeptides and as protein fusions. This combinatorial system of structural modules results in a large increase in diversity.

► **SXXK PBP fusions** of classes A and B are the lethal targets of β -lactam antibiotics. The PBP fusions of class A are comprised of an SXXK acyl transferase module of class A, linked to the carboxy end of a glycosyl transferase module having its own five motif-bar code, itself linked to the carboxy end of a membrane anchor [2]. They convert the disaccharide–pentapeptide units borne by lipid II precursor molecules into nascent polymeric (4–3) peptidoglycans. Glycan chain elongation is catalysed by the transglycosylase module. Peptide cross linking between elongated glycan chains is then carried out by the associated SXXK acyl transferase module. The PBP fusions of class B are comprised of an SXXK acyl transferase module of class B, bound to the carboxy end of a linker module having its own three-motif bar code, itself linked to a membrane anchor. They are components of morphogenetic apparatus that controls wall expansion, ensures cell-shape maintenance and carries out septum formation. Likely, the linker modules ensure that the



β-Lactam Antibiotics. Figure 2 (4–3) Peptidoglycan (the synthesis of which is susceptible to β-lactam antibiotics) and (3–3) peptidoglycan (the synthesis of which is resistant to antibiotics) in *Escherichia coli* and *Mycobacterium tuberculosis*. G: *N*-acetylglucosamine. M: *N*-acetylmuramic acid (i.e., *N*-acetylglucosamine with a *D*-lactyl substituent on carbon C₃). A₂pm: *meso*-diaminopimelic acid. In *E. coli* and *M. tuberculosis*, the stem peptides are unbranched. COX: COOH (in *E. coli*) or CONH₂ (in *M. tuberculosis*).

associated SXXK acyl transferase modules are positioned in an active conformation within the morphogenetic apparatus where they need to be. Binding of β -lactam antibiotics to the SXXK acyl transferase modules of the PBP fusions leads to the formation of Henri–Michaelis complexes that exhibit ligand- and enzyme-specific hydrogen bonding networks. *Escherichia coli* is killed in a number of ways; via cell lysis as a result of the selective inactivation of the class A PBP1a and PBP1b (which can substitute for each other) by cephaloridine and cefsulodin; via transformation of the cells into round bodies as a result of the selective inactivation of the cell-cycle subclass B2 PBP2 by mecillinam and thienamycin; via cell filamentation as a result of the selective inactivation of the cell-cycle subclass B3 PBP3 by mezlocillin, cefaperazone, cefotaxime, cefuroxime, cephalothin and aztreonam; or via different combinations of these morphological alterations by ampicillin, benzylpenicillin, carbenicillin and cefoxitin.

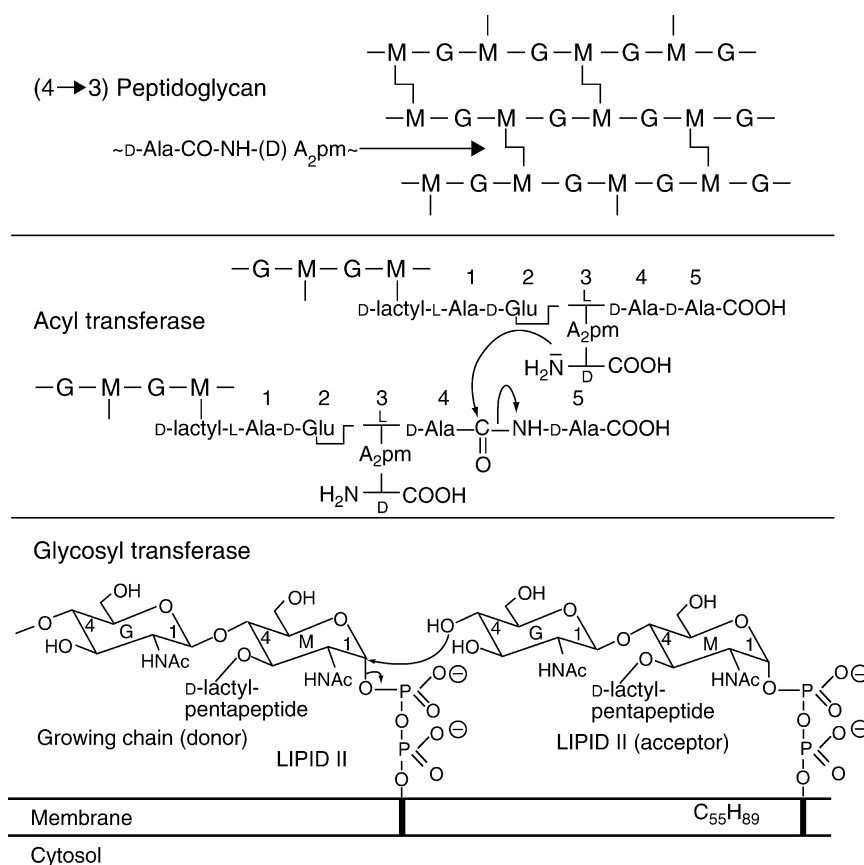
β -Lactamase-Mediated Resistance

► **SXXX free-standing PBPs** are peptidoglycan-hydrolases of one kind or another. Loss of these auxilliary cell-cycle proteins causes varying morphological aberrations, but is not fatal at least in the laboratory environment. Similarly, conversion of free-standing PBPs into β -lactam antibiotic-hydrolysing enzymes, with loss of peptidase activity and conservation of the polypeptide fold, gives rise to the **SXXX β -lactamases**. There are three classes of SXXX β -lactamases A, C and D, which are easily distinguished on the basis of their primary structures, although the tertiary structures are

clearly similar and also related to those of the acyl transferase modules of PBPs. In particular, in addition to the characteristic SXXK, motifs reminiscent of the bar code SXN and KTG groups are found in nearly superimposable positions. The catalytic pathway of β -lactamases follows the 3-step model shown above but with good substrates, the k_3 value can be higher than 1000 s^{-1} [4].

The class A enzymes have M_r values around 30,000. Their substrate specificities are quite variable and a large number of enzymes have emerged in response to the selective pressure exerted by the sometimes abusive utilization of antibiotics. Some of these “new enzymes” are variants of previously known enzymes, with only a limited number of mutations (1–4) but a significantly broadened substrate spectrum while others exhibit significantly different sequences. The first category is exemplified by the numerous TEM variants whose activity can be extended to third and fourth generation cephalosporins and the second by the NMCA and SME enzymes which, in contrast to all other SXXX β -lactamases, hydrolyse carbapenems with high efficiency. Despite these specificity differences, the tertiary structures of all class A β -lactamases are nearly superimposable.

The class C enzymes have M_r values of 39,000 and exhibit more uniform properties. They hydrolyse benzyl- and phenoxyethyl penicillin relatively well (turn-over numbers of 20–70 s^{-1}), ampicillin and amoxicillin 10- to 20-fold less rapidly and extremely poorly the other penicillins (generally due to low k_3 values). The early cephalosporins (cephalothin,



β -Lactam Antibiotics. Figure 3 Lipid II precursor (bottom) and polymeric (4–3) peptidoglycan of *Escherichia coli* and *Mycobacterium tuberculosis*. Glycosyl transferase and acyl transferase-catalysed reactions. G: *N*-acetylglucosamine. M: *N*-acetylmuramic acid. A₂pm: *meso*-diaminopimelic acid. (3–3) Peptidoglycan cross linking (Fig. 2) may proceed via the formation, in a penicillin-resistant manner, of a *N*-acyl-L-diaminoacyl moiety linked as an ester to the serine residue and the transfer of the peptidyl moiety to the ω -amino group of the diamino acid residue of another peptide.

cephalexin, cefazolin) are well hydrolysed but the later generation compounds also exhibit low to very low k_3 values, as do imipenem and aztreonam. A few mutations are known to extend the spectrum of some enzymes but the most currently utilized bacterial strategy is overproduction by deregulation of the biosynthetic control mechanism, so that the MICs of poor or even very poor substrates can increase significantly [5].

Class D enzymes (M_r of about 27,000) exhibit a high activity versus isoxazolyl penicillins, such as oxacillin and are referred to as the OXA-family. Surprisingly, the amino group of the SXXK lysine residues is carboxylated in the most active forms of the enzymes. Penicillins are generally better substrates than cephalosporins but mutations have been found which confer extended activity spectra to these enzymes.

Inactivators of class A β -lactamases (clavulanate, sulbactam, tazobactam) are themselves β -lactams and act as suicide substrates. They can be used in

combination with β -lactamase sensitive compounds (ampicillin, amoxicillin) but are not clinically useful against class C and class D producers. Some TEM mutants have also been selected that exhibit reduced sensitivity to these inactivators.

The class B metallo- β -lactamases have emerged more recently as a clinical problem but they are particularly dangerous since many of them hydrolyse all known β -lactams, with the exception of monobactams. In particular, they hydrolyse the suicide substrates mentioned above, as well as carbapenems that usually escape the activity of all the SXXK enzymes, with the exception of the NMCA group.

Three subclasses B1, B2 and B3 can be distinguished on the basis of the sequences. B1 and B3 enzymes are optimally active with 2 Zn²⁺ ions, while the B2 enzymes are inhibited by the second Zn²⁺. These B2 enzymes also exhibit a very narrow activity spectrum and only hydrolyse carbapenems. The 3D structures of representative members of each subfamily have been

solved and they highlight a typical αββα fold, completely unrelated to that of the SXXK enzymes. The production of β-lactamases can be inducible or constitutive and the genes carried by the chromosome or plasmids, some genes being parts of integrons. Several clinical strains produce up to three distinct enzymes.

PBP-Mediated Resistance

There are at least two modes of intrinsic resistance to β-lactam antibiotics. Determinants conferring a decreased susceptibility to β-lactam antibiotics evolve by the accumulation of point mutations in genes that code for essential PBP fusions of classes A and/or B. The shuffling and capture of DNA sequences from commensal *Streptococci* having a reduced susceptibility to the drug, give rise to *Streptococcus pneumoniae* pathogens in which mosaic genes code for mosaic PBP fusions of classes A and/or B of decreased affinity for the drug. Mosaic and wildtype PBP fusions of the same class (and the same subclass) differ by up to 15% amino acid residues. Mosaic PBPs occur also in *Neisseria meningitidis* and *Neisseria gonorrhoeae* strains.

Other strategies leading to an increased resistance are the transfer of a complete gene encoding a resistant PBP from a non-pathogenic related species to yield the Methicillin Resistant *Staphylococcus aureus* (MRSA) and the overproduction by *Enterococci* of a pre-existing but minor resistant PBP, which can further mutate to even more resistant forms. Some bacteria can also manufacture a (3–3) peptidoglycan (Fig. 2) with the help of a penicillin-resistant LD-transpeptidase, which is not an SXXK enzyme. Indeed, a low proportion of (3–3) cross links have been found in the walls of *Enterococcus faecium*, *Mycobacterium tuberculosis* and *Mycobacterium leprae*. The quantitative influence of these cross links on the resistance level remains to be determined but the synthesis of (3–3) peptidoglycan, combined with that of a set of relatively resistant SXXK PBPs and the limited permeability of the mycolic acid layer might explain the lack of efficiency of β-lactam antibiotics as therapeutic agents against tuberculosis and leprosy [6].

Outer Membrane Permeability and Active Efflux Systems

In Gram-negative bacteria, diffusion of β-lactam antibiotics into the periplasm (where the activity of PBPs takes place) occurs via the channels that porins create in the outer membrane. The number and properties of the porin molecules are such that diffusion is relatively rapid in *E. coli* but much slower in *Enterobacter* and *Pseudomonas*. Mutants can be selected after the permeability of porin channels or their number has been decreased. A slow diffusion into

the periplasm becomes a particularly important factor when it is combined with the presence of a β-lactamase (even in low concentration or when poorly active) in the same cellular compartment. Note that in *Mycobacteria*, the mycolic acid layer plays a role similar to that of the outer membrane in Gram-negatives. Finally, β-lactams bearing a hydrophobic side-chain can be ejected from the periplasm by the active efflux systems AcrAB-TolC in *E. coli* and *Salmonella typhimurium* and MexAB-OprM in *Pseudomonas*. Overexpression of these proteins can significantly increase the level of resistance.

Clinical Use

Because the SXXK PBPs are specific to the prokaryotes, the β-lactam antibiotics have a high selective toxicity without marked side effects except for possible allergic reactions. Resistance is a problem of great concern. The use of antibiotics fuels the continuing emergence and spreading of novel β-lactamases and intrinsic resistance determinants among bacterial pathogens.

► Microbial Resistance to Drugs

► Quinolones

► Ribosomal Protein Synthesis Inhibitors

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β-Lactamases

► β-Lactam Antibiotics.

Lateral Hypothalamic Area

The lateral hypothalamic area has been identified as a feeding centre by studies involving electric stimulation and discrete lesions. Neurons in the lateral hypothalamic area and the neighbouring perifornical area express neuropeptides that stimulate feeding when injected into cerebral ventricles (orexins 1 and 2, melanin-concentrating hormone (MCH)).

► Appetite Control

Laxatives

Laxatives, also called purgatives or cathartics, are substances used to hasten the transit of food through the intestine. Laxatives function by different mechanisms. Bulk laxatives, like methylcellulose or bran, contain agents like polysaccharide polymers, which are not fermented by the normal processes of digestion. They retain water in the gut lumen and promote bowel movements. Osmotic laxatives consist of poorly absorbable solutes such as salines containing magnesium cations or phosphate anions or non-digestible sugars and alcohols (glycerin, lactulose, sorbitol or mannitol). Osmotic laxatives retain an increased volume of fluid in the lumen of the bowel by osmosis, which accelerates the transfer of the bowel contents. Faecal softeners alter the consistency of the faeces. They are also called emollients and contain surface-active compounds similar to detergents, like docusate salts. Stimulant laxatives/purgatives increase the motility of the gut (peristalsis) and stimulate water and electrolyte secretion by the mucosa. Stimulant laxatives, which can cause abdominal cramps and deterioration of intestinal function, include diphenylmethane derivatives (bisacodyl, phenolphthalein), anthraquinone laxatives (derivatives of plants such as aloe, cascara or senna) and ricinoleic acid.

LCAT

► Lecithin-Cholesterol Acyltransferase

LD₅₀

► Lethal Dose

LDL-Cholesterol

► Low-Density-Lipoprotein

LDL-Receptors

LDL-receptors are receptors on the cell surface that remove LDL (and some other forms of) lipoproteins from the plasma into the cell.

► HMG-CoA-Reductase Inhibitors (Statins)
 ► Lipoprotein Metabolism
 ► Low-Density Lipo-protein Receptor Gene Family

Lead

A lead is a hit compound that displays specificity and potency against a target in a library screen and continues to show the initial positive dose-dependent response in more complex models such as cells and animals.

Lead Discovery by NMR

► SAR-by-NMR

Lecithin-Cholesterol Acyltransferase

Enzyme that converts free cholesterol to cholesteryl ester on HDL.

► Lipoprotein Metabolism

Leonardo

►14-3-3 Proteins

Leptin

The cytokine leptin is secreted by adipocytes (fat cells) in proportion to the size of the adipose depot and circulates via the bloodstream to the brain, where it ultimately affects feeding behavior, endocrine systems including reproductive function and, at least in rodents, energy expenditure. The major effect of Leptin is on the hypothalamus, where it suppresses appetite and hence food intake. Leptin exerts its effects via binding to the leptin receptor in the brain (specifically in the hypothalamus), which activates the JAK-STAT Pathway.

- Appetite Control
- Adipokines

Lethal Dose

The lethal dose (LD₅₀) is a measure of the toxicity of a compound. In an LD₅₀ toxicity test, various doses of a drug are administered to groups of animals, and the mortality in each group is determined. The lethal dose 50 is the dose, which is lethal for 50% of a group of animals.

Leucin Zipper

A leucine zipper is a structural motif present in a large class of transcription factors. These dimeric proteins contain two extended alpha helices that “grip” the DNA molecule much like a pair of scissors at adjacent major grooves. The coiled-coil dimerization domain contains precisely spaced leucine residues which are required for the interaction of the two monomers. Some DNA-binding proteins with this general motif contain other hydrophobic amino acids in these positions; hence, this structural motif is generally called a basic zipper.

Leukotrienes

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Synonyms

►Cysteinyl leukotrienes; B-leukotriene

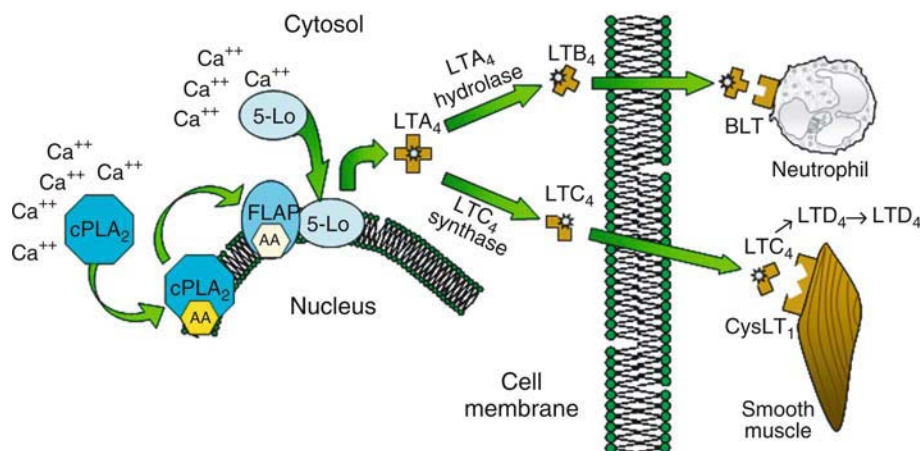
Definition

Leukotrienes belong to a large family of lipid mediators, termed eicosanoids, which are derived from arachidonic acid (AA) and released from the cell membrane by phospholipases. AA is subsequently converted by the enzyme, 5-lipoxygenase (5-LO) and a protein cofactor, ►5-lipoxygenase activating protein (FLAP), into two bioactive classes of leukotriene (LT): (i) LTB₄ and (ii) ►cysteinyl leukotrienes (cysLTs), LTC₄, D₄, and E₄. Leukotrienes do not exist in the state preformed in cells. These compounds play a significant role in allergic responses and contribute variably to the bronchoconstrictor response in human ►bronchial asthma, and possibly, chronic obstructive pulmonary disease.

Basic Characteristics

Synthesis and Metabolism

The site of synthesis of leukotrienes is largely or completely in the nuclear membrane. LTs are produced from cell membrane phospholipid-associated arachidonic acid (AA), which is liberated by 85 kDa cytosolic group IVAPLA₂ (cPLA₂) (Fig. 1) or phospholipase C (not shown) in response to cell activation. The secretory phospholipase group V PLA₂ (gVPLA₂) also is capable of inducing leukotriene synthesis, probably from phosphatidylcholine-rich membrane hydrolysis at both plasma or perinuclear membrane [1]. AA is converted to 5-HETE and subsequently to an unstable epoxide LTA₄ ([5(s)-trans-5,6-oxido-7,9-trans11,14-cis-eicosatetra-*n*onic acid]), a central intermediary in LT biosynthesis. LTs are synthesized in the cells by 5-LO which acts in concert with FLAP, a protein essential for 5-LO to function enzymatically in intact cells. In neutrophils, LTA₄ is transformed by LTA hydrolase to dihydroxy LTB₄, whereas in ►eosinophils, macrophages, basophils, and mast cells that express LTC₄ synthase, the terminal enzyme involved in cysLT synthesis, LTA₄ is conjugated with the tripeptide glutathione to form LTC₄. Outside of the cell, LTC₄ is rapidly converted by γ-glutamyltranspeptidase into LTD₄. LTD₄ is converted more slowly into LTE₄ by a dipeptidase, which is secreted into the urine and is sometimes used (controversially) as a marker for cysLT synthesis. Leukotriene B₄, a noncysteine



Leukotrienes. Figure 1 Schema representing cellular biosynthesis of cysteinyl leukotrienes (cysLTs) and BLT at the nuclear membrane. In this schema, cPLA₂ has migrated from the cytosol to the nuclear envelope and 5-LO is secreted from the nucleus to bind with FLAP and produce the metabolite, LTA₄, which is synthesized by 5-LO from 5-HPETE (not shown) in two steps. LTA₄ is converted by LTC₄ synthase which after LTC₄, is converted rapidly to LTD₄ outside of the inflammatory cell. Note that a specific hydrolase (LTA₄ hydrolase) also catalyzes the formation of LTB₄, a non-cysLT, which has its own receptor. See text for details.

containing dihydroxy leukotriene, binds to its own BLT receptor. The cysLTs are characterized by a side chain containing 3, 2, or 1 amino acid(s) (cysteine is always present); there are two or more specific cysLT receptors in tissues at various sites including airway smooth muscle, vascular smooth muscle, and inflammatory cells themselves. Both cysLT receptors have been characterized and cloned. LTD₄ binds to high affinity CysLT₁ receptor while CysLT₂ receptor is most preferred target for LTC₄.

Recent investigations have suggested that cysLT may also be synthesized in the cytosol in lipid bodies that are formed during cellular activation in eosinophils [2]. It has been suggested that all enzyme and transport systems essential to cysLT synthesis exist in these lipid bodies, and hence synthesis could occur without translocation of cPLA₂ to the nuclear envelope. Likewise, it has been suggested that 5-LO is not stored within the nucleus, but rather is an enzyme dispersed throughout the cytosol, which migrates to the perinuclear membrane and cytosolic lipid bodies upon cellular activation. Recently, the role of 14 kDa secretory PLA₂ in leukotriene synthesis has been explored in human granulocytes [1]. Studies demonstrated that gVPLA₂ is capable of causing AA synthesis and subsequent LT secretion by both cPLA₂-dependent and -independent pathways. Among the secretory enzymes, gVPLA₂ is unique because it can bind cell membranes by two distinct mechanisms: (i) cell surface proteoglycan through a cluster of cationic residues at the carboxy terminus of the enzyme and (ii) direct interfacial binding to membrane phosphatidylcholine. In eosinophils, gVPLA₂ is internalized and binds to perinuclear membrane to generate AA and subsequent

translocation of cytosolic 5-LO to perinuclear membrane. Accordingly, LTC₄ secretion caused by gVPLA₂ in eosinophils does not involve ERK-1/2 mediated cPLA₂ phosphorylation. By contrast, LTB₄ secretion caused by gVPLA₂ in neutrophils is mediated through activation of the cPLA₂ pathway. These novel pathways for gVPLA₂ are fully established in granulocytes; however, the role of other 14 kDa sPLA₂ isoforms in LT synthesis *in vitro* remains an area of considerable interest. sPLA₂s have been identified in the bronchoalveolar lavage fluid of asthmatic subjects.

Leukotriene Receptors

There are two distinct receptor types for LTB₄ (BLT₁ and BLT₂ receptors) and two for cysLTs (CysLT₁ and CysLT₂ receptors). Classification is based mainly on functional data as derived from recent cloning and biochemical characterization. For LTB₄, high affinity BLT₁-receptor shows high degree of specificity for LTB₄ (K_d 0.15–1 nM) and is only expressed in inflammatory cells. Recently identified low affinity BLT₂-receptor has a higher K_d value for LTB₄ (23 nM) and is expressed ubiquitously, in contrast to BLT₁, in leukocytes.

The cysLTs are a family of potent bioactive lipids that act through two structurally divergent G protein coupled CysLT₁ and CysLT₂ receptors. mRNA expression demonstrates the CysLT₁ gene, which is located on the X chromosome and is identified in macrophages, smooth muscle cells, leukocytes, lung, and spleen. The CysLT₁ receptor was originally identified on the basis of its contractile properties for bronchial smooth muscle. The agonist for the CysLT₁ receptor are

LTD₄ > LTC₄ > LTE₄. The second receptor, CysLT₂, binds equally to LTD₄ and LTC₄; mRNA is expressed in heart, leukocytes, spleen, brain, placenta, and lymph nodes. To date, the functional role of CysLT₂ receptor remains unclear. The current nomenclature is summarized in Table 2, together with order of potency of the LTs, names of some selective antagonist drugs and expression in human and mouse tissues/cells.

Role of Leukotrienes in Inflammatory Diseases

LTB₄, a B-leukotriene, is a potent chemotaxin for neutrophils and T-cells and a weak chemotaxin for human eosinophils. In human neutrophils, LTB₄ causes translocation of intracellular calcium that initiates an autocrine pattern of stimulated cellular activity. It promotes the adhesion of neutrophils to the vascular endothelium and enhances the migration across the endothelial wall into the surrounding tissues. LTB₄ also causes the release of toxic oxygen radicals, lysosomal enzymes, and chemokines/cytokines from proinflammatory cells. Some studies have shown that LTB₄ may cause contraction of both human bronchus and guinea pig parenchymal strips *in vitro*; however, blockade of LTB₄ receptor has little or no therapeutic action in human asthma.

The cysLTs were formerly known as SRS-A (slow reacting substance of anaphylaxis) because of the slow, sustained contraction of airway smooth muscle that resulted from the secretion of this substance(s) upon mast cell activation. Human bronchi may have a homogenous population of CysLT₁ receptors, whereas guinea pig trachea have both CysLT_{1/2} receptors. Although originally identified in tissue mast cells, eosinophils, and mast cells, both have substantial cysLT synthetic capacity. Because the eosinophil is ubiquitous in asthma and allergic disease and because it is capable of cysLT synthesis, this cell has generally been regarded as primary mechanism for sustained cysLT secretion in allergic states (Table 1). However, recent publications have suggested the eosinophil secretion is relatively minimal compared to mast cell secretory capacity for cysLTs.

Asthmatic exacerbations are associated with chemotaxis of eosinophils from the peripheral blood across endothelial membranes into the parenchyma and lumen of the conducting airways of the lung. This process is facilitated by the upregulation of β_1 - and β_2 -integrins, which bind to counterligands of the immunoglobulin supergene family on the endothelial surface. Diapedesis is facilitated by the presence of these ligands on both sides of the endothelium (luminal and parenchymal). The migration of eosinophils from the peripheral blood to human airways provides a continuous reservoir for leukotriene synthesis in allergic and asthma reactions. The 85 kDa cPLA₂ that catalyzes the first steps of leukotriene synthesis also serves as a messenger protein for cellular adhesion, likely by the synthesis of lysophospholipid, which is the by-product of membrane hydrolysis in which AA is also synthesized.

Leukotrienes play a role in both allergy and asthma [5]. While cysLTs are highly efficacious bronchoconstricting agents when administered exogenously to human asthmatics (Fig. 2) (1000-fold the potency of histamine), a substantial role for cysLT in asthmatic bronchoconstriction has not been established. Amelioration of inflammatory response by corticosteroids in both asthma and allergic reactions is highly efficacious and does not appear to result from blockade of leukotriene synthesis. No other chronic inflammatory disease has been linked directly to cysLT secretion despite the capacity for these compounds to cause edema and inflammatory cell migration.

Drugs

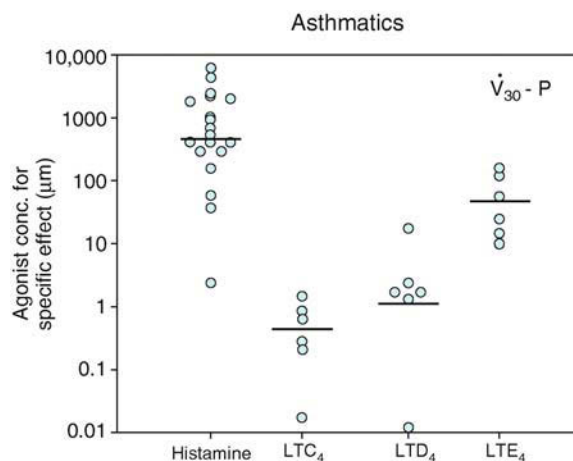
The effects of leukotrienes can be blocked at several levels. Inhibitors of FLAP or 5-LO inhibit LT synthesis at all levels. However, FLAP antagonists developed to date have been too hepatotoxic for human use. Zileuton, a 5-LO synthase inhibiting drug, also demonstrated some hepatotoxicity in a small percentage of patients, which was nonetheless entirely reversible. However, the short half-life of this compound requires four times daily

Leukotrienes. Table 1 Pharmacologic actions of the leukotrienes

LTB ₄	Cysteinyl LTs (LTC ₄ , LTD ₄ , LTE ₄)
PMN aggregation	Airway smooth muscle contraction
PMN chemotaxis	Constriction of conducting airways
Nuclear transcription	Contraction of guinea pig parenchyma
Exudation of plasma	Secretion of mucus
Translocation of calcium	Fluid leakage from venules
Stimulation of PLA ₂ (guinea pig)	Edema formation
Contraction of human bronchus (?)	Chemoattraction of eosinophils
Contraction of guinea pig parenchyma (?)	Autocrine activation of PLA ₂ (guinea pig)
No effect with LTRA	Blocked by LTRA

Reprinted with permission from [3].

administration, and accordingly, this compound is rarely prescribed. The most widely used antiLT drugs are the ►leukotriene receptor antagonists (LTRAs; see Table 2), which were synthesized before the cloning of the CysLT₁ receptor. These include pranlukast, which is widely



Leukotrienes. Figure 2 Relative airway responses of asthmatic patients to inhaled cysteinyl leukotrienes versus histamine. CysLTs have up to 1,000-fold greater potency in causing bronchoconstriction than histamine. Reprinted with permission from (2).

used in Japan, and zafirlukast and montelukast, which are used in the USA, Europe, and Asia. In controlled studies in human asthmatics, these compounds cause 5–8% improvement in bronchoconstriction as measured by the forced expiratory volume in 1 s (FEV₁). LTRAs have been shown to reduce the need for inhaled corticosteroids (ICS) and β -adrenoceptor agonists in human asthma. Additional studies have suggested that addition of LTRA to ICS or β -adrenoceptor agonists augments the improvement in FEV₁. Nonetheless, LTRAs are fairly expensive and substantially less efficacious than either long-acting β -adrenergic agonists (LABA) or ICS, particularly when LABA and ICS are used in combination. Accordingly, the use of LTRAs is often relegated to either mild asthma or as supplemental therapy for patients failing to respond to other drugs.

There are few definitive data to substantiate the efficacy of LTRA therapy in refractory asthma, except for patients with “aspirin-sensitive asthma.” This is a fairly uncommon form of asthma that occurs generally in adults who often have no prior (i.e., childhood) history of asthma or ►atopy, may have nasal polyposis, and who often are dependent upon oral corticosteroids for control of their asthma. This syndrome is not specific to aspirin but is provoked by any inhibitors of the cyclooxygenase-1 (COX-1) pathway. These patients have been shown to have a genetic defect that causes

Leukotrienes. Table 2 Leukotriene receptor type [4]

Nomenclature	BLT ₁ receptor	CysLT ₁ receptor (LTD ₄ receptor)	CysLT ₂ receptor (LTC ₄ receptor)
Relative potency of agonists	LTB ₄ > 12(R)-HETE (cysLTs are inactive)	LTD ₄ = LTC ₄ > LTE ₄ ^a	LTC ₄ > LTD ₄ > LTE ₄ ^a
Chromosome	Human: 14q11.2–q12 Mouse: 14	Human: Xq13.2–21.1 Mouse: X-D	Human: 13q14.12–q21.1 Mouse: 14-D1
Selective antagonists (IC ₅₀)	LY-255883 (>10 nM) CP-195543 (570 nM) U-75302 (1 μ M) CP-105696 (58 nM) ZK-158252 (260 nM)	Montelukast (MK476) ICI198615 Pobilukast (SFK104353) Zafirlukast (ICI204219) Pranlukast (ONO1078)	none
Expression (Human)	<i>Tissues:</i> Leukocytes, thymus, spleen, liver, ovary <i>Cells:</i> PBLs, neutrophils, T-cells, dendritic cells, mast cells, eosinophils, macrophages, leukocytes	<i>Tissues:</i> spleen, small intestine, placenta, lung smooth muscle, <i>Cells:</i> bronchial smooth muscle, CD34 ⁺ hemapoietic progenitor cells, monocytes, macrophages, mast cells, eosinophils, neutrophils, PBLs, human umbilical vein endothelial cells	<i>Tissues:</i> heart, skeletal muscle, spleen, brain, lymph node, adrenal medulla, lung, human pulmonary/saphenous vein <i>Cells:</i> monocytes, macrophages, mast cells, eosinophils, cardiac muscle, coronary artery, PBLs
Expression (Mouse)	<i>Tissues:</i> lungs, <i>Cells:</i> myeloid leukocytes, neutrophils, T-cells, macrophages, mast cells, eosinophils	<i>Tissues:</i> lung, skin, small intestine <i>Cells:</i> macrophages, fibroblasts, leukocytes	<i>Tissues:</i> lung, skin, brain, small intestine, spleen <i>Cells:</i> macrophages, fibroblasts, endothelial cells, leukocytes

^aPartial agonist in some tissues.

overexpression of the enzyme LTC₄ synthase. However, the mechanism by which bronchoconstriction is provoked by COX-1 inhibition remains unexplained. In such patients, LTRAs are specifically indicated. There is limited evidence suggesting that patients with aspirin-sensitive asthma can use selective COX-2 inhibitors safely; however, COX-2-specific analgesics still are not recommended for use in aspirin-sensitive asthma by the FDA in the USA and may themselves be cardiotoxic.

Although some studies have shown no change in the excretion of LTE₄ in the urine of patients treated with corticosteroids, other investigations indicated that corticosteroids inhibit cPLA₂ translocation to the nuclear membrane in inflammatory cells, thus attenuating stimulated synthesis of cysLTs. This would suggest that oral and possibly inhaled corticosteroids may also inhibit production of cysLTs both directly (see above) and indirectly by causing necrosis and apoptosis of eosinophils.

In the USA, LTRAs have largely replaced theophylline as the incremental drug for the treatment of moderate and severe asthma, where LABA plus ICS alone do not provide adequate control. For patients with mild persistent asthma, LTRAs have been designated as a suitable substitute for low dose ICS by the National Asthma Education Panel Program (NAEPP) of the National Heart and Lung Institute (National Institutes of Health). However, inhaled ICS are more efficacious.

Therapeutic Response to Antileukotriene Drugs

When given acutely to patients who have no prior exposure to LTRA therapy, FEV₁ increases measurably within 30 min and results in a maximal improvement of about 8% in 1 h. For short acting drugs (e.g., zileuton), this response returns rapidly to baseline, and the drug must initially be administered in four doses daily. In this regard, the initial effects of 5-LO inhibition are more like bronchodilators than disease modifying agents. However, with prolonged use (>60 days), there is little difference between peak and trough response in FEV₁, even if zileuton administration is decreased to twice daily. This acquired, longer acting effect has been suggested to imply a disease modifying effect of antileukotriene therapy. In some studies, even acute administration of LTRAs has caused a modest decrease in eosinophil migration into asthmatic airways and the presence of the cysLT receptor, which has been recently identified on eosinophils, suggests a possible mechanism for this action. Other recent studies indicate that the LTRA, montelukast, if infused intravenously causes rapid incremental increase in FEV₁ in patients seeking treatment for acute asthma. The reason for the improved efficacy by intravenous infusion is unclear, but further investigations are examining the future role of LTRAs as potential rescue drugs in acute asthma.

LTRAs are extremely safe for patient use. However, the present generation of LTRAs is only modestly efficacious. Many patients show no clinically meaningful response, and current recommendations suggest a 1 month trial period to determine if patients will benefit from these drugs. With the exception of aspirin-sensitive asthmatics, there is currently no means for predicting which patients or under what circumstances antileukotriene therapies will be effective.

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Levodopa

- L-DOPA / Levodopa
- Dopamine System
- Anti-Parkinson Drugs

Lewy Bodies

Lewy bodies are typical in neuronal degeneration, which is accompanied by the presence of these eosinophilic intracellular inclusions of 5–25 µm diameter in a proportion of still surviving neurons. Lewy bodies contain neurofilament, tubulin, microtubule-associated proteins 1 and 2, and gelsolin, an actin-modulating protein.

- Anti-Parkinson Drugs

LH-RH Agonist

LH-RH (Luteinizing Hormone-Releasing Hormone) agonists are drugs that inhibit the secretion of sex hormones. In men, LH-RH agonists cause testosterone levels to fall. In women, LH-RH agonists cause the levels of estrogen and other sex hormones to fall.

- ▶ Contraceptives
- ▶ Targeted Cancer Therapy

Liddle's Syndrome

Liddle's syndrome is an autosomal dominant disorder that is caused by persistent hyperactivity of the epithelial Na channel. Its symptoms mimic aldosterone excess, but plasma aldosterone levels are actually reduced (pseudoaldosteronism). The disease is characterized by early onset arterial hypertension, hypokalemia, and metabolic alkalosis.

Disease-causing mutations are found in the cytoplasmic regulatory region of the β and γ subunits of the epithelial sodium channel (ENaC) genes. In general, patients with Liddle's syndrome can be treated successfully with the ENaC inhibitor amiloride.

- ▶ Diuretics
- ▶ Epithelial Na^+ Channels

Ligand

A ligand can be an antagonist or agonist that binds to a receptor.

Ligand-Gated Ion Channels

Ion channels that are opened by binding of a neurotransmitter (or drug) to a receptor domain on extracellular sites of the channel protein(s) are defined

as ligand-gated. Nicotinic acetylcholine, glutamate, γ -aminobutyric acid_A (GABA_A) and glycine receptors are examples of this type of receptor-linked ion channel.

- ▶ Benzodiazepines
- ▶ Ca^{2+} Channels
- ▶ Ionotropic Glutamate Receptors
- ▶ Nicotinic Receptors
- ▶ Non-selective Cation Channels
- ▶ K^+ Channels
- ▶ Serotonergic System
- ▶ Purinergic System
- ▶ Table Appendix: Receptor Proteins
- ▶ Transmembrane Signaling
- ▶ Glycine Receptor
- ▶ GABAergic System

Limbic System

Collection of interconnected subcortical and cortical brain structures (including hypothalamus, amygdala, and hippocampus) integrating multimodal intero- and exteroceptive information to produce coherent neuro-endocrine and behavioral output, and to support memory functions.

- ▶ Orexins

Linkage Disequilibrium (LD)

Polymorphisms in the human genome are often not independently transmitted; i.e., a polymorphism is associated with particular variants present on the same chromosome. Recombination erodes this association, but for physically close polymorphisms (e.g., within a gene), the correlation, known as LD, persists over time.

- ▶ Pharmacogenomics

Lipid-lowering Drugs

Lipid-lowering drugs are drugs that affect the lipoprotein metabolism and that used in therapy to lower plasma lipids (cholesterol, triglycerides). The main classes of

drugs used clinically are statins (HMG-CoA reductase inhibitors), anion exchange resins (e.g. cholestyramine and cholestipol), fibrates (bezafibrate, gemfibrozil or clofibrate) and other drugs like nicotinic acid.

- ▶ HMG-CoA-Reductase-Inhibitors
- ▶ Fibrates
- ▶ Anion Exchange Resins

Lipid Metabolism

- ▶ Lipoprotein Metabolism

Lipid Modifications

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Synonyms

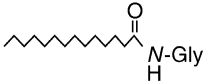
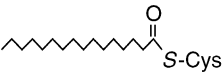
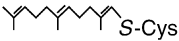
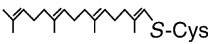
Lipidation; *S*-acylation; *N*-myristoylation; Myristoylation; *S*-prenylation; Prenylation; Palmitoylation; Isoprenylation; ▶ **GPI anchors**; Glypiation

Definition

Covalent attachment of lipid moieties to proteins plays important roles in the cellular localization and function of a broad spectrum of proteins in all eukaryotic cells. Such proteins, commonly referred to as lipidated proteins, are classified based on the identity of the attached lipid (Fig. 1). Each specific type of lipid has unique properties that confer distinct functional attributes to its protein host. *S*-acylated proteins, commonly referred to as palmitoylated proteins, generally contain the 16-carbon saturated acyl group palmitoyl attached via a labile thioester bond to cysteine residue(s), although other fatty acyl chains may substitute for palmitoyl group. *N*-myristoylated proteins contain the saturated 14-carbon myristoyl group attached via amide bond formation to amino-terminal glycine residues. *S*-prenylated proteins contain one of two ▶ **isoprenoid** lipids, either the 15-carbon farnesyl or 20-carbon geranylgeranyl. The fourth major class of lipidated proteins is those containing the glycosylphosphatidylinositol (GPI) moiety, a large and complex structure of which the lipid component is an entire phospholipid.

Basic Mechanisms

S-acylated proteins include many GTP-binding regulatory proteins (G proteins), including most α subunits of ▶ **heterotrimeric G-proteins** and also many members of the Ras superfamily of monomeric G proteins, a number of G protein-coupled receptors, several non-receptor ▶ **tyrosine kinases**, and a number of other signaling molecules. *S*-acylation is posttranslational and reversible, a property that allows the cell to control

Lipid	Structure	Position of modification
<i>N</i> -myristoyl		Amino-terminal glycine
<i>S</i> -acyl (<i>S</i> -palmitoyl)		Cysteine, no defined consensus
<i>S</i> -prenyl (farnesyl)		Cysteine at/near carboxyl-terminus
<i>S</i> -prenyl (geranylgeranyl)		Cysteine at/near carboxyl-terminus
GPI anchor	Complex structure includes phosphatidylinositol and sugars	Carboxyl-terminus

Lipid Modifications. Figure 1 Major classes of lipid-modified proteins.

the modification state, and hence the localization and biological activity, of the protein [1]. The lipid substrates for *S*-acylation are ►acyl-CoA molecules. The molecular mechanism of *S*-acylation is only recently being elucidated; the first identified acyltransferase specifically involved in the process being the product of the *Skinny Hedgehog* (*Ski*) gene in *Drosophila*. *Ski* orthologs have also been identified in mammalian genomes. This acyltransferase appears to specifically process secreted proteins, in particular a molecule important in development termed Hedgehog.

The process by which cytoplasmic proteins are subject to *S*-acylation is also becoming clarified. The breakthrough in this arena came from genetic screens in yeast, in which two related *S*-palmitoyltransferases were uncovered that are termed Erf2 and Akr1. These two gene products share a common sequence referred to as a DHHC (aspartate-histidine-histidine-cysteine) motif, and a large number of genes encoding DHHC-motif proteins can be identified in all eukaryotes for which genome information is available. Although biochemical details of the enzymatic function of DHHC-motif proteins are still rather limited, the available evidence is consistent with the DHHC domain playing a direct role in the protein acyltransferase reaction.

In addition, nonenzymatic acylation of cysteine thiols on proteins incubated in the presence of acyl-CoA has been described, although the biological importance of this process is still unclear.

N-myristoylated proteins include select α subunits of heterotrimeric G proteins, a number of nonreceptor tyrosine kinases, a few monomeric G-proteins, and several other proteins important in biological regulation [2]. There is some overlap between *S*-acylated and *N*-myristoylated proteins such that many contain both lipid modifications. Such “dual lipidation” can have important consequences, most notably the localization of the dually modified species to distinct membrane subdomains termed ►lipid rafts or ►caveolae. *N*-myristoylation is a stable modification of proteins. The myristoyl moiety is attached to the protein cotranslationally by the enzyme myristoyl CoA: protein *N*-myristoyltransferase (NMT); the lipid substrate is myristoyl-CoA. NMT has been extensively studied in regard to substrate utilization and kinetic properties, and crystal structures of fungal enzymes are available. There are two distinct but closely related genes encoding NMTs in mammalian cells, *NMT1* and *NMT2*, although differences between the two isoforms have not yet been described.

S-prenylation is the most recent of the four major types of lipid modifications to be described. As with *S*-acylation, *S*-prenylation is posttranslational. The lipid substrates for these modifications are farnesyl diphosphate and geranylgeranyl diphosphate. The mechanism

involves attachment of the isoprenoid lipid to cysteine residues at or near the carboxyl terminus through a stable thioether bond [3]. Two distinct classes of *S*-prenylated proteins exist in eukaryotic organisms. Proteins containing a cysteine residue fourth from the carboxyl terminus (the so-called CaaX-motif) can be modified by either the 15-carbon farnesyl or 20-carbon geranylgeranyl isoprenoid by one of two closely related termed protein farnesyltransferase (FTase) and protein geranylgeranyltransferase type 1 (GGTase-1); these enzymes are collectively referred to as CaaX prenyltransferases [4]. FTase and GGTase-1 are $\alpha\beta$ heterodimers; the α subunits of the enzymes are identical while the β subunits are the products of distinct, but related, genes. The identity of the “X” residue of the CaaX motif dictates which of the two enzymes recognize the substrate protein. Following prenylation, most CaaX-type proteins are further processed by proteolytic removal of the three carboxyl-terminal residues (i.e., the -aaX) by the Rce1 CaaX protease and methylation of the now-exposed carboxyl group of the prenylcysteine by the Icmt methyltransferase. A number of *S*-prenylated proteins are also subject to *S*-acylation at a nearby cysteine residue to produce a dually lipidated molecule, although this type of dual modification does not apparently target the protein to the same type of membrane subdomain as the dual acylation noted above.

The second class of *S*-prenylated proteins is a member of the Rab family of proteins, which is involved in membrane trafficking in cells. These proteins are geranylgeranylated at two cysteine residues at or very near to their carboxyl-terminus by protein geranylgeranyltransferase type 2 (GGTase-2), also known as Rab GGTase [5]. GGTase-2 is also a $\alpha\beta$ heterodimer and its subunits show significant sequence similarity to the corresponding subunits of the CaaX prenyltransferases [6]. However, unlike the CaaX prenyltransferases, monomeric Rab proteins are not substrates for GGTase-2. In order to be processed by the enzyme, newly synthesized Rab proteins first bind and form a stable complex with a protein termed Rep, and it is the Rab–Rep complex that is recognized by GGTase-II. The enzyme acts in a processive fashion, attaching both geranylgeranyl groups to closely spaced cysteine residues at or near the carboxyl-terminus of the Rab proteins. All three of the protein prenyltransferases (FTase, GGTase-1, GGTase-2) have been extensively characterized in regard to substrate recognition, mechanism, and structure.

GPI-anchored proteins constitute a quite diverse family of cell-surface molecules that participate in such processes as nutrient uptake, cell adhesion, and membrane signaling events [3]. All GPI-linked proteins are destined for the cell surface via trafficking through the secretory pathway, where they acquire the

preassembled GPI moiety. The entire procedure, which involves assembly of the GPI moiety from ►**phosphatidylinositol** and sugars and proteolytic processing of the target protein to expose the GPI addition site at the carboxyl-terminus of the protein, involves a number of gene products. Many of the genes associated with GPI biosynthesis have been cloned by complementation of GPI-deficient mammalian cell lines and temperature-sensitive yeast GPI mutants. The precise mechanisms through which these enzymes work in concert to produce a GPI-anchored protein are just now beginning to be elucidated.

Pharmacological Intervention

Since the mechanisms of protein *S*-acylation are so poorly understood, there is little in the way of good pharmacological agents that target this process. One compound that has been used with modest success is cerulenin, which apparently mimics the acyl-CoA substrate in the reaction catalyzed by the putative *S*-acyltransferase that acts on intracellular proteins. In contrast, there has been substantial effort to identify and characterize specific inhibitors of *N*-myristoylation [2]. Genetic and biochemical studies have established NMT as a target for development of anti-fungal drugs. The enzyme is also a potential target for the development of antiviral and antineoplastic agents. Both peptidic and nonpeptidic inhibitors of NMTs, particularly fungal NMTs, have been described.

There has been enormous effort in the past 10 years to develop pharmacological agents targeting the *S*-prenylation by CaaX prenyltransferases, especially FTase [4]. This is primarily due to the interest in one subset of *S*-prenylated proteins, the Ras proteins, due to the important role of Ras in ►**oncogenesis**. Ras proteins are modified by the 15-carbon farnesyl isoprenoid, and farnesylation of these proteins is indispensable for both normal biological activity and oncogenic transformation. Selective inhibitors of FTase, termed FTIs, can reverse Ras-mediated oncogenic transformation of cells, and several are in clinical development as anticancer therapeutics. Literally hundreds of potent inhibitors of FTase, and many of GGTase-1, have been identified using several strategies, including design of analogs of the CaaX peptide and isoprenoid substrates and by high-throughput screening of natural product and compound libraries. These compounds can be placed into four distinct categories: mimics of CaaX tetrapeptides, mimics of FPP, ►**bisubstrate analogs**, and organic compounds selected from natural product and chemical libraries. There has been relatively little development of pharmacological agents targeting GGTase-2. It is likely, however, that some of the isoprenoid analogs that show activity against GGTase-1 will also have inhibitory activity on GGTase-2 given that both enzymes use the same isoprenoid substrate.

There is also increasing interest in developing pharmacological agents targeting the biosynthesis of GPI-anchored proteins [3]. Such proteins are particularly abundant on the surface of a number of protozoan organisms. Several devastating tropical diseases such as African sleeping sickness and Chagas disease are caused by protozoan parasites that rely heavily on cell-surface GPI-anchored proteins for both inhabiting their host and escaping immune detection. In studies primarily involving gene disruption approaches, several of the enzymes involved in GPI biosynthesis have been identified as attractive targets for development of antiparasitics. To date, there is very little publicly available information on specific inhibitors of the enzymes involved in GPI biosynthesis and attachment, but it is likely that such information will be forthcoming.

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Lipid Phosphate Phosphohydrolases

Lipid phosphate phosphohydrolases (LPPs), formerly called type 2 phosphatidate phosphohydrolases (PAP-2), catalyse the dephosphorylation of bioactive phospholipids (phosphatidic acid, ceramide-1-phosphate) and lysophospholipids (lysophosphatidic acid, sphingosine-1-phosphate). The substrate selectivity of individual LPPs is broad in contrast to the related sphingosine-1-phosphate phosphatase. LPPs are characterized by a lack of requirement for Mg^{2+} and insensitivity to *N*-ethylmaleimide. Three subtypes (LPP-1, LPP-2, LPP-3) have been identified in mammals. These enzymes have six putative transmembrane domains and three highly conserved domains that are characteristic of a phosphatase superfamily. Whether LPPs cleave extracellular mediators or rather have an influence on intracellular lipid phosphate concentrations is still a matter of debate.

Lipid Rafts

Lipid rafts are specific subdomains of the plasma membrane that are enriched in cholesterol and sphingolipids; many signaling molecules are apparently concentrated in these subdomains.

►Lipid Modifications

Lipid Transfer Proteins

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Synonyms

Plasma lipid transfer proteins

Definition

Plasma lipid transfer proteins, which include the cholesteryl-ester-transfer-protein (CETP; previously known as lipid transfer protein I, LTP-I) and the phospholipid-transfer-protein (PLTP; previously known as lipid transfer protein II, LTP-II) mediate the transfer of lipids (cholesteryl esters, triglycerides and phospholipids) between lipoproteins present in human plasma. These proteins significantly affect plasma lipoprotein concentration and composition.

Basic Characteristics

Gene and Protein Characteristics

The genes coding for CETP and PLTP belong to one gene family, which also includes lipopolysaccharide-binding-protein (LBP) and bactericidal/permeability-increasing-protein (BPI). This common descent not only becomes clear from a considerable sequence similarity (45–65% homology at the cDNA level), but also from substantial conservation of exon/intron transitions.

The gene encoding CETP is located on the long arm of chromosome 16 (16q12–21), spans approximately 25 kbp and harbors 16 exons and 15 introns. The molecule mass of its mature protein product is 74 kDa. Major sites of CETP gene expression in humans are the

liver, spleen and adipose tissue, with lower levels of expression in the small intestine, adrenals, kidney and heart. CETP present in human plasma seems to originate predominantly from the liver and adipose tissue, although data that have assessed this directly in man are lacking. The majority of human plasma CETP (~90%) is bound to ►**high-density lipoprotein** (HDL) particles. An important regulatory factor of CETP gene expression is hypercholesterolemia, which induces gene transcription. Sequences necessary for this cholesterol-responsiveness are located in the natural flanking regions of the human CETP gene, upstream of the transcription start site. This regulatory sequence concerns an element of two direct repeats separated by four nucleotides (DR4 element). This element enhances CETP gene expression following activation through a heterodimer, containing the transcription factors Liver X Receptor (LXR) and Retinoid X Receptor (RXR). In order to enhance gene transcription, these transcription factors require activation through ligands, which include cholesterol and its oxysterols.

The gene coding for PLTP has been mapped to chromosome 20 (20q12–13.1), where also the genes for LBP and BPI are situated. The PLTP gene contains 13.3 kbp, with similar organization as compared to CETP (16 exons–15 introns). Purified PLTP has a molecule mass of 81 kDa. PLTP is expressed in a variety of tissues including placenta, pancreas, lung, kidney, heart, liver, adipose tissue, skeletal muscle and brain. Macrophages also express PLTP and may contribute significantly to plasma PLTP activity levels. PLTP gene expression can be upregulated by cholesterol in a similar way as described for CETP. In addition, PLTP gene expression is subject to regulation via response elements for the peroxisome proliferator activated receptor (PPAR) alpha and the farnesoid X-activated receptor (FXR).

Detailed protein structures have been reported for BPI and CETP. Given the aforementioned similarities within this gene family, these protein structures serve as a likely model for the protein structure of PLTP. CETP and BPI are elongated molecules, shaped like a boomerang. There are two domains with similar folds, and a central beta-sheet domain between these two domains. The molecules contain two lipid-binding sites, one in each domain near the interface of the barrels and the central beta-sheet.

Function

In humans, CETP and PLTP are directly involved in the transfer of lipids between different ►**lipoprotein** classes. Through their action, these lipid transfer proteins have major effects on the concentration and composition of HDL. This section further describes the physiological function of CETP and PLTP in humans.

CETP mediates the exchange of cholesteryl esters and triglycerides between HDL and the proatherogenic,

apoB-containing lipoprotein fractions, predominantly ►**very-low density lipoprotein** (VLDL). Specifically, triglycerides are transported from VLDL to HDL, with cholesteryl esters transferred in the opposite direction. It is generally acknowledged that CETP itself has no preference for the type of lipoprotein in its substrate specificity. The VLDL–HDL axis is likely to result from the presence of plasma lipid transfer inhibitor protein (LTIP), which is associated almost exclusively with ►**low-density lipoprotein** (LDL) and inhibits the CETP-mediated transfer of cholesteryl esters and triglycerides. LTIP suppresses the involvement of LDL, thereby directing the CETP activity towards the remaining lipoprotein fractions, VLDL and HDL. The CETP-mediated lipid exchange is an equimolar and energy neutral process. The exchange seems to occur by a shuttle mechanism, rather than by the formation of a complex between CETP and donor and acceptor lipoproteins. In addition to LTIP, composition and concentration of lipoprotein substrates are important regulatory factors of the CETP-mediated exchange activity, and under physiological conditions, the net mass of VLDL-triglycerides may be rate limiting. The relevance of CETP for human lipid metabolism became evident after the identification of humans with partial or near complete loss of CETP activity due to the presence of a mutation in one or both alleles of the CETP gene. In these subjects, who predominantly live in Japan, (partial) loss of plasma CETP reduces the ability of cholesteryl esters to be transported out of HDL. This results in a net increase in plasma HDL cholesterol (HDL-C) levels, with the appearance of HDL particles that have increased in size. This change is paralleled by increased concentrations of ►**apolipoprotein A-I** (apoA-I), which results from delayed catabolism of these large sized HDLs. In some cases of severe CETP deficiency, the reduced transfer of cholesteryl esters from HDL to VLDL furthermore gives also rise to mildly decreased levels of LDL cholesterol (LDL-C), which originates from VLDL. Recently, the first Caucasian family was described with partial CETP deficiency due to a novel splice site mutation in the CETP gene.

PLTP is responsible for the majority of phospholipid transfer activity in human plasma. Specifically, it transfers surface phospholipids from VLDL to HDL upon lipolysis of triglycerides present in VLDL. The exact mechanism by which PLTP exerts its activity is yet unknown. The best indications for an important role in lipid metabolism have been gained from knockout experiments in mice, which show severe reduction of plasma levels of HDL-C and apoA-I. This is most likely the result of increased catabolism of HDL particles that are small in size as a result of phospholipid depletion. In addition to the maintenance of normal plasma HDL-C and apoA-I concentration, PLTP is also involved in a process called HDL conversion. Shortly summarized, this cascade of processes leads to fusion of HDL

particles into larger particles, with a concomitant release of lipid-poor apoA-I. This newly formed lipid-poor apoA-I is a pivotal particle in the uptake of free cholesterol from peripheral cells via efflux through ABCA1. This is a potentially antiatherogenic function of PLTP, but – surprisingly – overexpression of human PLTP in mice results in increased susceptibility for diet-induced atherosclerosis and a reduction of plasma HDL-C, again due to hypercatabolism of apoA-I. At present, no reports exist on genetic PLTP deficiency in humans. Therefore, any hypothesis on the relevance of PLTP activity for human lipid metabolism remains to be confirmed. When it comes to humans, plasma PLTP activity is increased in insulin-resistant individuals with high plasma triglycerides and low HDL-C, as well as in patients with diabetes mellitus. In patients with type 2 diabetes, PLTP activity is positively associated with intima-media thickness, which is a surrogate measure for the risk of atherosclerotic disease. In addition, elevated PLTP activity is decreased by statin treatment.

Drugs

Plasma HDL-C concentration is negatively associated with occurrence of cardiovascular disease, independent of age, gender, LDL-C and other established risk factors. Consequently, there is great interest for pharmacological interventions to raise HDL-C. Among the drugs that are currently available, statins, fibrates and nicotinic acid exert such an increasing effect on plasma HDL-C concentrations. These drugs, however, are limited in their HDL-C raising efficacy (statins: +3% to +9%, fibrates: +0% to +11%, nicotinic acid: +7% to +23%) or exhibit significant side effects (nicotinic acid: flushes). The high levels of HDL-C observed in case of CETP deficiency have led to the development of strategies to raise HDL-C by inhibition of CETP activity in humans. Two such strategies are currently in advanced stage of development, i.e., vaccine-induced inhibition and inhibition of CETP activity through small-molecule compounds. Among these, the latter has attracted most attention. The effects of CETP inhibition through small-molecule compounds parallel those observed in genetic CETP deficient subjects to a large extent: increase of plasma HDL-C and apoA-I concentration, with the occurrence of large sized HDL particles due to cholesteryl ester enrichment. In case of potent CETP inhibition, plasma levels of LDL-C and apoB are also decreased. With respect to the effects on cardiovascular disease exerted by these lipoprotein changes, data from epidemiological studies might justify optimism. However, clinical trials assessing the consequences of CETP inhibition on surrogate and clinical cardiovascular endpoints are required. One such trial has recently been terminated due to an increased number of lethal cardiovascular events occurring in the CETP-inhibition group. At the moment,

it remains to be established whether this relates to intrinsic characteristics of the study drug or to the mechanism of CETP-inhibition in general.

Due to the absence of human models for PLTP deficiency, our knowledge about the relevance of plasma PLTP activity for human lipid metabolism is still incomplete. No investigational drugs are available that specifically target the activity of this protein.

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Lipidation

►Lipid Modifications

Lipopolysaccharide

A lipopolysaccharide (LPS) is any compound consisting of covalently linked lipids and polysaccharides. The term is used more frequently to denote a cell wall component from Gram-negative bacteria. LPS has endotoxin activities and is a polyclonal stimulator of B-lymphocytes.

►Neutrophils ►Inflammation

Lipoprotein Lipase

Endothelial anchored enzyme in muscle and adipose tissue primarily responsible for hydrolysis of chylomicron and VLDL triglycerides.

►Lipoprotein Metabolism

Lipoprotein Metabolism

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Synonyms

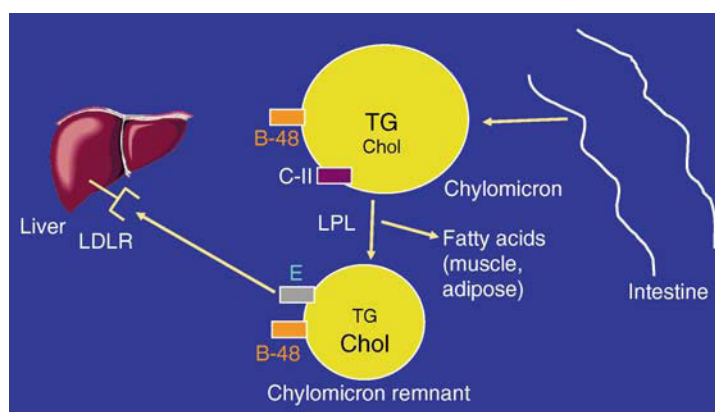
Lipid metabolism; Cholesterol metabolism

Definition

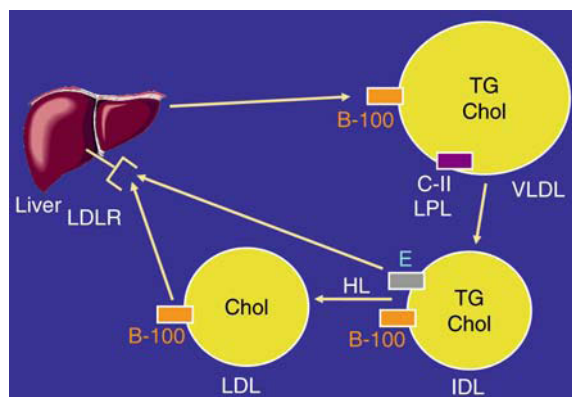
►**Lipoprotein** metabolism is the process by which hydrophobic lipids, namely triglycerides and cholesterol, are transported within the interstitial fluid and plasma. It includes the transport of energy in the form of triglycerides from intestine and liver to muscles and adipose, as well as the transport of cholesterol both from intestine and liver to peripheral tissues, as well as from peripheral tissues back to the liver.

Basic Mechanisms

Lipoproteins are large macromolecular complexes, which transport triglycerides and cholesterol within the blood. Triglycerides are a key component of energy transport and metabolism, and cholesterol is an essential component of all cells and required for steroidogenesis. The structure of lipoproteins is a hydrophobic neutral lipid core consisting of triglycerides and cholesteryl esters, surrounded by amphipathic phospholipids and specialized proteins known as ►**apolipoproteins**. Phospholipids serve to permit interaction with the aqueous environment, and apolipoproteins are required for the structural integrity of lipoproteins and direct their metabolic interactions with enzymes, lipid transport proteins, and cell surface receptors. The five major families of lipoproteins are ►**chylomicrons**, ►**very-low-density lipoproteins** (VLDL), ►**intermediate-density lipoproteins** (IDL), low-density lipoproteins (LDL), and ►**high-density lipoproteins** (HDL). Chylomicrons are the largest and most lipid-rich lipoproteins, whereas HDL are the smallest lipoproteins and contain the least amount of lipid. In the exogenous pathway of lipid transport (Fig. 1), dietary fat and cholesterol are absorbed by intestinal enterocytes and incorporated into chylomicrons, which contain the major structural apolipoprotein B-48. Chylomicrons are initially secreted into lymph thus bypassing the hepatic first pass effect. Once they gain entry into the systemic circulation they bind to ►**lipoprotein lipase** (LPL) on the luminal surface of the capillary endothelium of tissues (►**endothelial lipase**), especially muscle and adipose tissue. The LPL hydrolyzes the triglycerides (apolipoprotein C-II on the chylomicron surface is a required cofactor for LPL). The free fatty



Lipoprotein Metabolism. Figure 1 Exogenous pathway of lipoprotein metabolism.



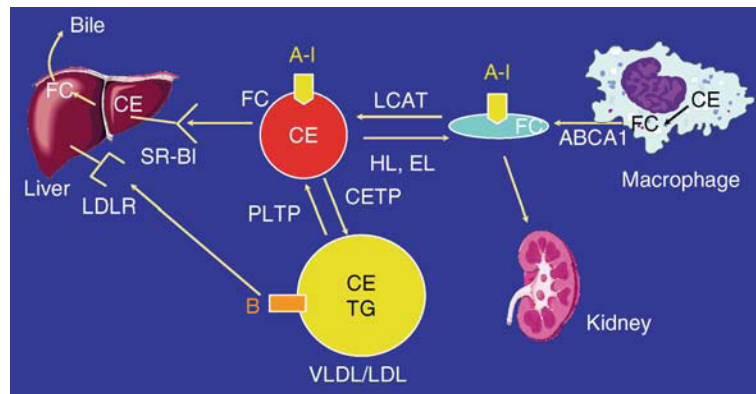
Lipoprotein Metabolism. Figure 2 Endogenous pathway of lipoprotein metabolism.

acids enter the tissue to be used for energy (muscle) or storage (adipose) and the triglyceride-depleted chylomicron remnant (CMR) is released. CMRs are taken up by the liver by binding of apolipoprotein E to the LDL receptor and the LDL receptor-related protein (LRP). In the endogenous pathway of lipid transport (Fig. 2), the liver synthesizes triglycerides and cholesteryl esters and packages them into VLDL, which contain the major structural apolipoprotein B-100. VLDLs are hydrolyzed by LPL to form intermediate density lipoproteins (IDL). IDL can be taken up by the liver via binding of apolipoprotein E to the LDL receptor or LRP. Alternatively, the triglyceride and phospholipid in IDL can be hydrolyzed by ▶**hepatic lipase** (HL) within the hepatic sinusoids to form LDL. LDL can be taken up by peripheral cells or by the liver by the binding of apolipoprotein B-100 to the LDL receptor.

The pathways of HDL metabolism and reverse cholesterol transport are complex (Fig. 3, [1]). HDL and its major apolipoprotein apoA-I are synthesized by both the intestine and the liver. A second major

HDL protein apoA-II is made only by the liver. Nascent HDL interacts with peripheral cells to facilitate the removal of excess free cholesterol through a process that is facilitated by the cellular protein ATP-binding cassette protein A1 (ABCA1). Some of the acquired free cholesterol is esterified to cholesteryl ester on the HDL particle by the action of the enzyme ▶**lecithin-cholesterol acyltransferase** (LCAT) and the nascent HDL particle becomes the larger HDL₃. HDL acquires further cholesteryl ester by continued LCAT action and eventually becomes the even larger HDL₂. HDL₂ can selectively transfer both cholesteryl ester and free cholesterol to the liver via an HDL receptor in the liver called scavenger receptor BI (SR-BI). Cholesteryl esters can also be transferred from HDL₂ to apoB-containing lipoproteins such as VLDL and LDL via the action of the ▶**cholesteryl ester transfer protein** (CETP) and then returned to the liver by hepatic uptake of LDL. HDL₂ triglycerides and phospholipids can be hydrolyzed by HL and endothelial lipase (EL) to remodel it to HDL₃. Cholesterol derived from HDL contributes to the hepatic cholesterol pool used for bile acid synthesis and the cholesterol is eventually excreted into the bile and feces as bile acid or free cholesterol.

Disorders of lipoprotein metabolism involve perturbations which cause elevation of triglycerides and/or cholesterol, reduction of HDL-C, or alteration of properties of lipoproteins, such as their size or composition. These perturbations can be genetic (primary) or occur as a result of other diseases, conditions, or drugs (secondary). Some of the most important secondary disorders include hypothyroidism, diabetes mellitus, renal disease, and alcohol use. Hypothyroidism causes elevated LDL-C levels due primarily to downregulation of the LDL receptor. Insulin-resistance and type 2 diabetes mellitus result in impaired capacity to catabolize chylomicrons and VLDL, as well as excess hepatic triglyceride and VLDL production. Chronic kidney disease, including but not limited to end-stage



Lipoprotein Metabolism. Figure 3 HDL metabolism and reverse cholesterol transport.

renal disease, is associated with moderate hypertriglyceridemia due to a defect in triglyceride lipolysis and remnant clearance. Nephrotic syndrome is associated with a more pronounced hyperlipidemia involving both elevated triglycerides and cholesterol due to hepatic overproduction of VLDL. Alcohol consumption inhibits oxidation of free fatty acids by the liver, which stimulates hepatic triglyceride synthesis and secretion of VLDL; the usual lipoprotein pattern associated with alcohol consumption is moderate hypertriglyceridemia.

Inherited disorders of lipoprotein metabolism have provided important insights into the molecular regulation of lipoprotein metabolism in humans. The familial hyperchylomicronemia syndrome (FCS) (also known as Type I hyperlipoproteinemia), characterized by severely elevated triglycerides, is caused by genetic deficiency of either $\blacktriangleright$ LPL or apoC-II, demonstrating the essential nature of each of these protein for the hydrolysis of chylomicron triglycerides. Familial dysbetalipoproteinemia (FD) (also known as Type III hyperlipoproteinemia), characterized by elevation in apoB-containing lipoprotein remnant particles containing both triglycerides and cholesterol, is caused by specific mutations in the gene for apolipoprotein E (apoE), most commonly homozygosity for the relatively common apoE2 variant. This proved the essential nature of apoE for normal clearance of lipoprotein remnants by the liver. Familial hypercholesterolemia (FH) (also known as Type IIa hyperlipoproteinemia), characterized by elevation in LDL cholesterol, is caused by loss-of-function mutations in the LDL receptor. Homozygotes are more severely affected than heterozygotes, demonstrating the gene dose effect of the LDL receptor on LDL-C levels. This disorder established the key role of the LDL receptor in regulating plasma levels of LDL-C through mediating uptake of LDL by the liver. Mutations in other genes have been subsequently discovered to regulate the hepatic expression of the LDL receptor and thus influence plasma LDL-C levels. These include

loss-of-function mutations in the *autosomal recessive hypercholesterolemia (ARH)* gene, a recessive trait, and gain-of-function mutations in the *proprotein convertase subtilisin/kexin type 9 (PCSK9)* gene, an autosomal dominant trait. Sitosterolemia is caused by mutations in one of two members of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family, ABCG5 and ABCG8, resulting in increased cholesterol in the liver and secondary downregulation of the LDL receptor. Finally, mutations in the receptor binding region of apoB-100, the ligand for the LDL receptor, can impair its binding to the LDL receptor and delay the clearance of LDL, a condition known as familial defective apoB-100 (FDB).

Genetic conditions causing low cholesterol have also provided important insights and novel pharmacologic targets. Abetalipoproteinemia is characterized by absence of plasma apoB-containing lipoproteins and is caused by mutations in the gene encoding microsomal triglyceride transfer protein (MTP), a protein that transfers lipids to nascent chylomicrons and VLDL in the intestine and liver, respectively. Familial hypobetalipoproteinemia is characterized by markedly reduced LDL-C and apoB levels and is caused by mutations in apoB that generally result in premature truncation that either reduce secretion and/or accelerate catabolism. Finally, loss-of-function mutations in *PCSK9* have been shown to cause low LDL-C levels and substantial lifetime protection from CHD. All three of these gene products are targets for the development of new therapies for reducing LDL-C levels.

Genetic disorders of HDL metabolism have also resulted in greater understanding of the molecular regulation of HDL metabolism. Nonsense or missense mutations in apoA-I can result in substantially reduced HDL-C levels due to rapid catabolism of structurally abnormal or truncated apoA-I proteins. Tangier disease is a rare autosomal codominant disorder characterized by markedly low HDL-C and apoA-I levels and caused

by mutations in the gene *ABCA1*. ABCA1 facilitates efflux of cholesterol from cells to lipid-poor apoA-I, and in its absence apoA-I is not appropriately lipidated and is rapidly cleared from the circulation. LCAT deficiency causes markedly low levels of HDL-C, establishing the importance of cholesterol esterification by LCAT in the maintenance of normal HDL metabolism. Finally, CETP deficiency causes markedly elevated levels of HDL-C, establishing the importance of cholesteryl ester by CETP in the maintenance of normal HDL metabolism. Again, all of these gene products are targets of new HDL-based therapies.

Pharmacologic Relevance

LDL Cholesterol Reduction

Disorders of lipoprotein metabolism are important risk factors for atherosclerotic vascular disease (ASCVD). Thus the field of lipoprotein metabolism has been a fertile area for the development of drugs that modulate the levels of plasma lipoproteins. Intervention with drugs to reduce **LDL** cholesterol has been proven to decrease the risk of cardiovascular events [2]. Intervention with drugs to reduce triglycerides or raise HDL-C has not yet been definitively proven to reduce cardiovascular events. The early clinical trials for LDL-C reduction utilized niacin, bile acid sequestrants, and even the surgical approach of partial ileal bypass to reduce serum cholesterol levels. However, the bulk of the data supporting LDL-C reduction comes from the clinical outcome trials with **HMG CoA-reductase** inhibitors (statins). Early studies focused on patients with preexisting CHD, including the Scandinavian Simvastatin Survival Study (4S), the Cholesterol and Recurrent Events (CARE) study, and the Long-Term Intervention with Pravastatin in Ischemic Disease (LIPID) trial and established the efficacy of statin therapy in reducing cardiovascular events in patients with CHD. Early primary prevention trials included the West of Scotland Coronary Prevention Study (WOSCOPS) and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) and confirmed that the benefits of LDL cholesterol reduction extend to the primary prevention setting. Subsequent studies have expanded downward the range of baseline LDL-C levels for which statin therapy is beneficial as well as the target LDL that is effective [2], and include the Heart Protection Study (HPS), the Anglo-Scandinavian Cardiac Outcomes Trial Lipid-lowering Arm (ASCOT-LLA), the Collaborative Atorvastatin Diabetes Study (CARDS), the Treat to New Targets (TNT) trial, and the Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT) study. These studies strongly support the “lower is better” approach to reducing LDL-C as applied to high-risk patients.

Thus drug therapy for LDL-C reduction is widely used. Statins are the cornerstone of LDL-C reducing drug therapy. By inhibiting cholesterol biosynthesis in the liver, statins lead to increased hepatic LDL receptor expression. There are six statins currently available: lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin, and rosuvastatin. There is wide interindividual variation in the initial response to a statin, but once a patient is on a statin, the doubling of the statin dose produces a very predictable 6% further reduction in LDL-C. Some patients treated with statins develop muscle fatigue or pain, and severe myopathy and rhabdomyolysis have been reported. The risk of statin-associated myopathy is increased by the administration of drugs that interfere with the cytochrome P450 metabolism of statins [3]. Due to both inadequate reduction of LDL-C in some patients as well as statin intolerance in others, there is a clinical need for LDL-reducing drug therapy beyond statins. The most commonly used class is that of cholesterol absorption inhibitors; currently ezetimibe is the only drug in this class clinically available. Ezetimibe binds to and inhibits the function of NPC1L1, a major cholesterol transporter in the intestinal enterocyte responsible for absorption of luminal cholesterol derived both from diet as well as from bile. The inhibition of intestinal cholesterol absorption reduces hepatic cholesterol content and results in upregulation of hepatic LDL receptor expression. Ezetimibe at the 10 mg dose reduces cholesterol absorption by about 60% and reduces LDL-C levels by about 18% on average as monotherapy, and to a similar extent when used in combination with a statin. Ezetimibe is used in combination with statins to further reduce LDL-C levels and in patients who are statin-intolerant. In some cases, a third class of LDL-lowering drugs, bile acid sequestrants, is needed. Bile acid sequestrants bind bile acids in the intestine, prevent their reabsorption, and accelerate their loss in the feces. In order to maintain an adequate bile acid pool, the liver diverts cholesterol to bile acid synthesis, resulting in decreased hepatic cholesterol and upregulation of hepatic LDL receptor expression. Cholestyramine and colestipol are insoluble resins that must be suspended in liquid and colessevalam is available as large tablets, and relatively large amounts of bile acid sequestrants must be used to achieve effective LDL reduction.

Inability to achieve LDL-C goals with existing drug therapy remains an important unmet medical need. Advances in our understanding of the molecular regulation of LDL metabolism have generated new pharmacologic targets. These include the following: (i) inhibition of squalene synthase, another important enzyme in the cholesterol biosynthetic pathway; (ii) inhibition of MTP, which reduces VLDL production

and has been shown to reduce LDL-C levels; (iii) inhibition of apoB production using an antisense oligonucleotide approach; (iv) inhibition of PCSK9 based on the persuasive genetic data reviewed above.

The data supporting reduction in triglycerides or raising of HDL-C are much less abundant [4]. Fibrates and nicotinic acid are the most effective drugs in lowering TGs and raising HDL-C. Fibrates are agonists of PPAR α and are most effective as triglyceride lowering drugs, with modest effects in raising HDL-C. Fibrates lower triglycerides by activating PPAR α to stimulate ►LPL (enhancing triglyceride hydrolysis) and reduce hepatic apoC-III synthesis (enhancing clearance of TG-rich lipoproteins). A limited number of clinical trials with fibrates support the concept that they may reduce cardiovascular risk, particularly in patients with the phenotype of insulin-resistance. Increasingly, fibrates are being used in combination with statins. Nicotinic acid, or niacin, is a B-complex vitamin that in high doses lowers triglycerides and raises HDL-C levels. Niacin acutely reduces free fatty acid levels through inhibition of adipocyte triglyceride lipolysis, an effect now known to be mediated by the niacin receptor GPR109A, a G protein coupled receptor primarily expressed on adipocytes. Whether this mechanism accounts for the triglyceride lowering and HDL-C raising associated with niacin therapy is uncertain. The clinical use of niacin is limited by prostaglandin-mediated cutaneous flushing which is also mediated by GPR109A expressed on dermal macrophages. Data that niacin reduces cardiovascular risk is limited, but are consistent with a cardioprotective effect. Patients with combined hyperlipidemia may achieve their LDL-C goal with a statin alone, but frequently have persistently elevated triglycerides and often do not achieve their non-HDL-C goal. In this situation, the addition of either niacin or a fibrate to the statin can be highly effective in reducing the triglycerides and non-HDL-C levels. The therapy of low HDL-C is a major unmet medical need and new therapies designed to raise HDL-C levels or promote HDL function are under active development [1].

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Lipoproteins

The main transport form of lipids in the circulation. They are spherical macromolecules of 10–1200 nm diameter composed of a core of neutral lipids (mostly cholesterol ester and triglycerides) surrounded by an amphipathic shell of polar phospholipids and cholesterol. Embedded in the shell of lipoproteins are apolipoproteins that are essential for assembly of the particles in tissues that secrete lipoproteins, and for their recognition by target cells.

- Lipoprotein Metabolism
- Lipid Transfer Proteins
- HMG-CoA-Reductase Inhibitors
- Low-Density Lipoprotein Receptor Gene Family

Liposomes

Phospholipid vesicles, uncoated or polyethylenglycol-coated. They can be used to vehicle drugs, antibodies or nucleic acids to target cells.

5-Lipoxygenase

5-lipoxygenase is the enzyme causing catalysis of arachidonic acid into leukotriene A₄.

- Leukotrienes

Lipoxygenases

- Leukotrienes

Lithocholic Acid

3 α -Hydroxy-5 β -cholanoic acid, a hepatotoxic and cholestatic secondary bile acid, formed by bacterial dehydroxylation of primary bile acids in the intestine.

- Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes

Local Anaesthetics

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Definition

Local anaesthetics are drugs that reversibly interrupt impulse propagation in peripheral nerves thus leading to autonomic nervous system blockade, analgesia, anaesthesia and motor blockade in a desired area of the organism.

Mechanism of Action

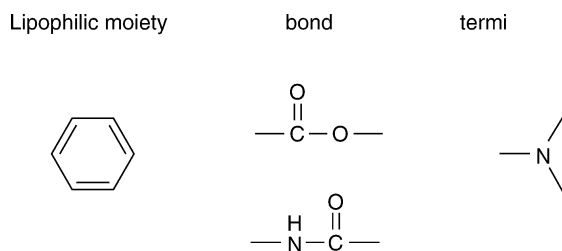
Impulse propagation in the ►peripheral nervous system depends on the interplay between ion channels selective to potassium and sodium. Briefly, few voltage insensitive potassium channels allow diffusion of positively charged potassium ions from the internal side of the axon to the exterior. This leaves a negative charge at the internal side that is called resting potential and amounts to approximately -80 mV over the ►axonal membrane. Upon a small depolarisation of the membrane, ►voltage-gated Na^+ channels open (activate) and conduct positively charged sodium ions from the exterior to the interior of the axon that depolarises the membrane to about $+60$ mV (action potential). After a few milliseconds the channels close spontaneously (inactivation) terminating the action potential and thus giving it an impulse like character. The action potential spreads electrotonically to neighbouring sodium channels that also open to produce an action potential. In this way, the impulse is propagated along the nerve to (afferent) or from (efferent) the central nervous system. Local anaesthetics inhibit ionic current through voltage gated sodium channels in a concentration dependent and reversible manner therefore directly blocking the impulse propagation process in either direction [1]. The interaction between the local anaesthetic molecule and the sodium channel is complex. The binding affinity is low for resting channels, but dramatically increases when the channel is activated. Thus, at high stimulus frequency sodium current block increases (use-dependent block). Use-dependent block is not important for conduction block during local anaesthesia since very high concentrations are reached locally producing complete and sufficient block. However, when local anaesthetics such as lidocaine are applied intravenously, lower systemic concentrations may already induce use-dependent block and thus reduce excitability in electrically active cells. This mechanism is important for the successful use of lidocaine as an antiarrhythmic or analgesic drug.

The putative binding site for local anaesthetic molecules at the sodium channel has been identified as two amino acids in the sixth membrane-spanning segment of domain IV [2]. This binding site is located directly underneath the channel pore and can only be reached from the internal side of the membrane. Because local anaesthetics are applied exterior to the nerve fibre, they have to penetrate the axonal membrane before they can bind to the channel.

Besides sodium channels, other ion channels such calcium- and potassium channels as well as certain ligand-gated channels are affected by local anaesthetics. However, this plays only a minor role for nerve block but may have more impact on adverse effects induced by systemical concentrations of these drugs.

Structure Activity Relation

Local anaesthetics comprise a lipophilic and a hydrophilic portion separated by a connecting hydrocarbon chain (Fig. 1). The hydrophilic group is mostly a tertiary amine such as diethylamine; the lipophilic group is usually an unsaturated aromatic ring such as xylylidine or para-aminobenzoic acid. The lipophilic portion is essential for anaesthetic potency whereas the hydrophilic portion is required for water solubility. Both groups are important for binding the drug molecule to the sodium channel. The connecting hydrocarbon chain usually contains an ester or an amide, in rare cases also an ether, and separates the lipophilic and hydrophilic portions of the molecule in an ideal distance for binding to the channel. Local anaesthetics are classified by the nature of this bond into ester or amide local anaesthetics. This bond is also important for metabolism of the drugs and to adverse reactions such as allergic reactions. Clinically relevant local anaesthetics have an efficacy of 100%, i.e. at high concentrations they completely abolish the sodium current. Their blocking potencies range from a few micromolar to millimolar half-maximal blocking concentration and are highly dependent on lipid solubility. To a smaller amount potency also depends on the structure of the molecule and on the type of bonding



Local Anaesthetics. Figure 1 Common structure of local anaesthetics. A lipophilic moiety on the left, an aliphatic spacer containing an ester or amide bond in the middle and an amine group on the right are the typical structural elements for local anaesthetic drugs.

(Table 1). Further determinants of blocking potency are the membrane potential and the state in which the sodium channel is in (resting, activated, inactivated). The tertiary amine group can be protonated giving most local

anaesthetics a pK_a of about eight so that a larger amount of the drug is in the hydrophilic form when injected into tissue with physiological pH. However, only the unprotonated lipophilic form is able to penetrate the

Local Anaesthetics. Table 1 Examples of clinically used local anaesthetics

Name	Structure	Relative potency	Mean blocking duration [h]	Lipid solubility log[P]	Molwt. [g/mol]	pKa
Procaine		1	0.5–1	1.92	236	9.1
Tetracaine		8	2–3	3.73	264	8.6
Lidocaine		2	1–2	2.26	234	8.2
Prilocaine		2	1–2	2.11	220	7.9
Mepivacaine		2	1.5–2	1.95	246	7.9
Ropivacaine		6	3–5	2.90	274	8.2
Bupivacaine		8	4–6	3.41	288	8.2

The ending “caine” stems from cocaine, the first clinically employed local anaesthetic. Procaine and tetracaine are ester-linked substances, the others are amides. Amide bonded local anaesthetics usually contain two i's in their name, ester-bonded only one. In the structure drawings, the lipophilic portion of the molecule is depicted at the left, the amine at the right. The asterisk marks the chiral centre of the stereoisomeric drugs. Lipid solubility is given as the logarithm of the water:octanol partition coefficient, log(P).

axonal membrane, which is required before binding to the sodium channel can occur. In a very acidic environment, like inflamed tissue, local anaesthetics are highly protonated, therefore cannot penetrate the axonal membrane and have little effect.

Toxicity

Local anaesthetics interfere with all voltage-gated sodium channel isoforms in an organism and thus with all electrically excitable cells in organs such as brain, heart and muscle. The major unwanted effects of local anaesthetics are thus disturbances of brain and heart function occurring during systemically high concentrations after overdosing or after accidental intra-vascular injection. Cerebral convulsions, general anaesthesia as well as dysrhythmias up to asystolic heart failure or ventricular fibrillation are feared as rare but most harmful complications. Other adverse effects can be allergic reactions. This occurs especially with ester-bonded local anaesthetics because their metabolism produces para-aminobenzoic acid that may serve as a hapten. As a special case, the local anaesthetic prilocaine is metabolised to o-toluidine, which may induce methemoglobinemia, especially in patients that have a glucose-6-phosphatase deficiency. The ►**S-stereoisomers** of the piperidine local anaesthetics bupivacaine and ropivacaine have now been introduced into clinical practise to reduce side effects. Their blocking potencies are minimally lower compared to their R-counterparts but their therapeutic index is wider.

Clinical Use

Local anaesthetics are mainly employed to induce regional anaesthesia and analgesia to allow surgical procedures in a desired region of the organism. Nerve block result in autonomic nervous system blockade, analgesia, anaesthesia and motor blockade. The patient normally stays awake during surgical procedures under regional anaesthesia, but regional anaesthesia can also be combined with general anaesthesia to reduce the requirement of narcotics and analgesic drugs. Local anaesthetics have to be injected locally into the circumference of a peripheral nerve, which gives sufficiently high concentrations to achieve conduction block. Certain injection techniques and procedures such as single nerve block as well as spinal, ►**epidural**, or intravenous regional anaesthesia have evolved to achieve the desired nerve block. It is also possible to place a catheter adjacent to a nerve and continuously apply local anaesthetics to receive long-term analgesia. Low concentrations of lipophilic local anaesthetics may be used for differential nerve block, i.e. only block of sympathetic and nociceptive fibres whereas somatosensory and motor fibres are less affected. Differential nerve block for example is useful in labour analgesia or for analgesia after surgical procedures of the extremities.

After local anaesthetic injection, onset of nerve block and duration depends mainly on lipid solubility and on the region in where the drug is injected. In some formulations adrenaline is added to prolong the blocking action by inducing regional vasoconstriction and hereby reduce absorption and metabolism.

The amide local anaesthetic lidocaine may also be used as an antiarrhythmic for ventricular tachycardia and extrasystoles after injection into the blood circulation. Drugs with high lipid solubility such as bupivacaine cannot be used for these purposes because their prolonged binding to the channel may induce dysrhythmias or asystolic heart failure [3]. Systemically applied lidocaine has also been used successfully in some cases of ►**neuropathic pain syndromes** [4]. Here, electrical activity in the peripheral nervous system is reduced by used-dependent but incomplete sodium channel blockade.

►General Anaesthetics

►Voltage-dependent Na⁺ Channels

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Locus

A specific region within a DNA sequence.

►Pharmacogenomics

Locus Ceruleus

The locus ceruleus is a structure located on the floor of the fourth ventricle in the rostral pons. It contains more than 50% of all noradrenergic neurons in the brain, and projects to almost all areas of the central nervous system.

The locus ceruleus is important for the regulation of attentional states and autonomic nervous system activity. It has also been implicated in the autonomic and stress-like effects of opiate withdrawal. A noradrenergic pathway originating from the locus ceruleus which descends into the spinal cord is part of the descending inhibitory control system, which has an inhibitory effect on nociceptive transmission in the dorsal horn.

Long-Chain Fatty Acids

Long-chain fatty acids (LCFAs) are aliphatic compounds with a terminal carboxyl group and with a chain length greater than 12 carbon atoms (e.g., lauric acid). Very long-chain fatty acids are fatty acids with more than 18 carbon atoms (e.g., stearic acid).

► [Fatty Acid Transport Proteins](#)

Long-QT Syndromes

Long-QT syndromes (LQTS) are potentially fatal inherited cardiac arrhythmias characterized by prolonged or delayed ventricular repolarization, manifested on the electrocardiogram as a prolongation of the QT interval. LQTS can be caused by mutations of at least six genes including KCNQ1 for LQT1, KCNH2 for LQT2, SCN5A encoding a cardiac sodium channel for LQT3, KCNE1 for LQT5 and MiRP1 for LQT6. LQT4 is linked to the mutations located in chromosome 4q25–27. Blockade of HERG channels is the most commonly identified drug-induced LQT.

► [K⁺ Channels](#)

► [Voltage-dependent Na⁺ Channels](#)

Long-Term Depression

Long-term depression (LTD) is a synaptic plasticity phenomenon that corresponds to a decrease in the synaptic strength (decrease in the post-synaptic response observed for the same stimulation of the pre-synaptic

terminals) observed after a specific stimulation of the afferent fibres. This decreased response is still observed hours after its induction.

► [Metabotropic Glutamate Receptors](#)

Long-Term Potentiation

Synonyms

LTP

Definition

Long-term potentiation (LTP) is a synaptic plasticity phenomenon that corresponds to an increase in the synaptic strength (increase in the post-synaptic response observed for the same stimulation of the presynaptic terminals) observed after a high frequency stimulation (tetanus) of the afferent fibres. This increased response is still observed hours and even days after the tetanus. The phenomenon is often observed at glutamatergic synapses and involves, in most cases, the activation of the *N*-methyl *D*-aspartate (NMDA) subtype of ionotropic glutamate receptors.

► [Metabotropic Glutamate Receptors](#)

► [Nicotinic Receptors](#)

► [Ionotropic Glutamate Receptors](#)

Loop Diuretics

► [Diuretics](#)

Low-density-lipoprotein-cholesterol

LDL is the major carrier of cholesterol to the periphery and supplies the cholesterol essential for the integrity of nerve tissue, steroid hormone synthesis, and cell membranes. The association between elevated plasma cholesterol carried in LDL and the risk of coronary heart disease has been well established. LDL is also sometimes called the “bad” cholesterol.

► [Lipoprotein Metabolism](#)

Low-density Lipoprotein Receptor Gene Family

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Synonyms

Low-density lipoprotein receptor-related proteins; LRP

Definition

The low-density lipoprotein (►LDL) receptor gene family encompasses a group of structurally related endocytic receptors that are expressed in many mammalian and nonmammalian cell types. The receptors mediate cellular binding and uptake of a variety of diverse macromolecules including lipoproteins and lipid-carrier-complexes, proteases and protease inhibitors, signaling factors, as well as viruses and toxins. Gene family members play crucial roles in many biological processes ranging from lipid homeostasis, to signal transduction, to cell migration.

Basic Characteristics

Receptor-Mediated Endocytosis

Endocytic receptors are cell surface proteins that transport macromolecules into cells through a process known as receptor-mediated ►endocytosis. In this process, a receptor on the cell surface binds a specific ligand from the extracellular space, internalizes via specialized in-tucked regions of the plasma membrane called clathrin-coated pits, and moves to an intracellular vesicle (►endosome) to discharge its cargo. From endosomal compartments, ligands are further directed to lysosomes for catabolism, while the unliganded receptor returns to the cell surface to initiate the next round of endocytosis. Endocytic receptors serve to regulate the concentration of ligands in the extracellular space and to deliver them to cells in need of these metabolites. Binding of some ligands to endocytic receptor may also trigger intracellular signaling cascades involved in regulation of important physiological processes such as embryonic patterning, neuronal cell migration, or synaptic plasticity.

Structural Organization of the LDL Receptor Gene Family

Much of our knowledge of the structure and function of endocytic receptors is based on the analysis of the LDL receptor gene family. Members of this extended gene family can be found in a variety of species ranging from roundworms to insects, to vertebrates. Ten receptors exist in mammalian organisms, all of which share common structural motifs required for receptor-mediated

endocytosis (Fig. 1). Among other modules, their extracellular domains are composed of clusters of ►complement-type repeats, the site of ligand binding, as well as β -propellers, essential for pH-dependent release of ligands in endosomes. The cytoplasmic tails harbor recognition sites for cytosolic adaptor proteins involved in protein trafficking and signal transduction. All ectodomains share significant sequence similarity in line with the ability of the receptors to bind an overlapping spectrum of ligands. In contrast, their cytoplasmic domains are unique, indicating distinct cellular fates for macromolecules internalized by individual receptors [1].

Functions of the LDL Receptor Gene Family

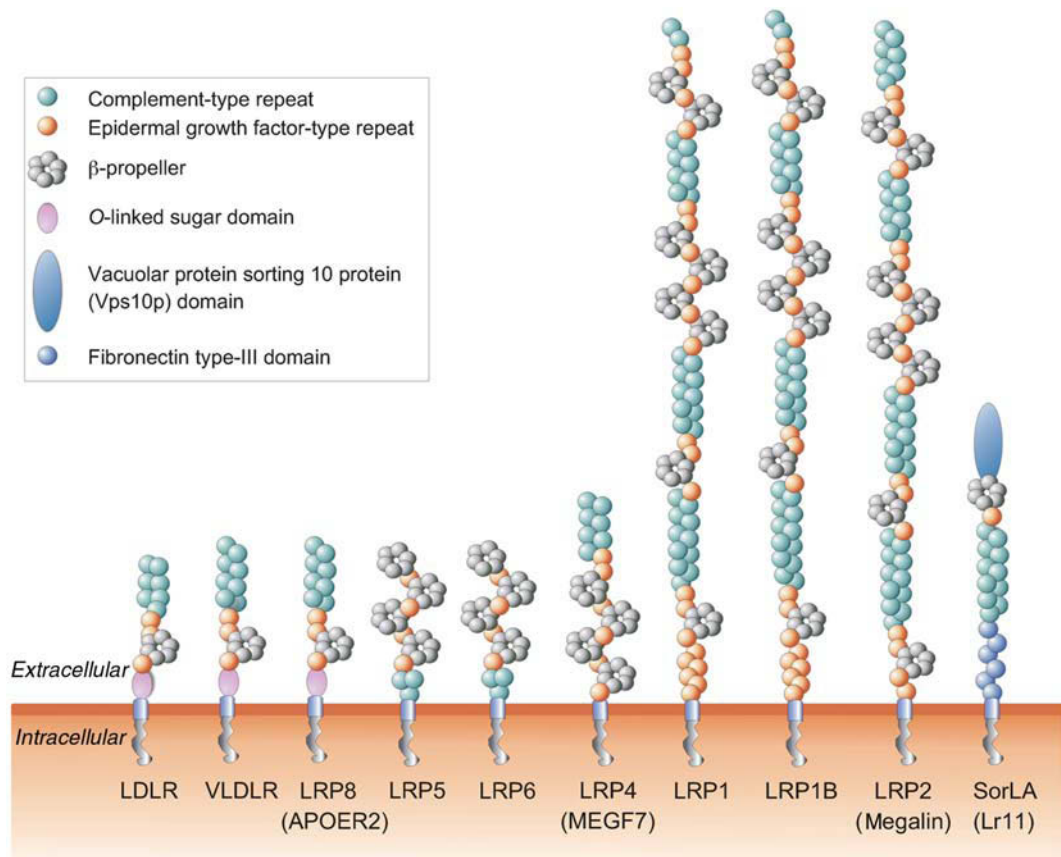
Transgenic animal models with spontaneous or induced receptor gene defects have been instrumental in elucidating the physiological roles of the LDL receptor gene family. In addition, a number of human diseases have been identified that are caused by sporadic or inherited forms of receptor deficiency (Table 1).

LDL Receptor

The LDL receptor is the archetype receptor of the gene family. It is the major endocytic receptor responsible for cellular uptake of cholesterol-rich ►lipoproteins from the circulation and extracellular body fluids. It binds lipoprotein particles that contain the ►apolipoproteins B-100 (APOB-100) or APOE, and mediates their endocytic uptake. Particles internalized via the LDL receptor are delivered to lysosomal degradation where the apolipoproteins are broken down into amino acids while the lipids are released into the cytosol for further metabolism. The uptake of lipoproteins by the LDL receptor has a dual role in lipid homeostasis. It delivers cholesterol required for maintenance of cellular functions (e.g., formation of membranes, synthesis of steroid hormones, and bile acids), and it regulates the concentration of this lipid in the circulation. The importance of the latter function is underscored by pathological abnormalities observed in patients with LDL receptor gene defects; a syndrome designated familial hypercholesterolemia (►FH). In humans with FH, lack of LDL receptor activity results in massive increase in circulating LDL. The mean plasma cholesterol levels are elevated approximately twofold in individuals with heterozygous and fourfold in patients with homozygous gene defects. As a consequence of fulminate ►hypercholesterolemia, FH patients suffer from premature atherosclerosis and coronary artery disease. To date more than 150 different mutations have been identified in the human LDL receptor gene.

VLDLR and APOER2

Very low-density lipoprotein receptor (►VLDLR) and APOE receptor-2 (►APOER2) are two gene family members with redundant functions. They are expressed in neurons of the developing brain and act as cell



Low-density Lipoprotein Receptor Gene Family. Figure 1 The LDL receptor gene family. The figure depicts the structural organization of mammalian members of the LDL receptor gene family. APOER2 (apolipoprotein E receptor 2); LDLR (low-density lipoprotein receptor); LRP (LDL receptor-related protein); MEGF7 (multiple epidermal growth factor repeat-containing protein 7); sorLA (sorting protein-related receptor); VLDLR (very low-density lipoprotein receptor). Designations in brackets indicate alternative names for the respective receptors.

Low-density Lipoprotein Receptor Gene Family. Table 1 Human diseases of the LDL receptor gene family

Receptor	Type of mutation	Disease
LDL re-ceptor	Loss-of-function (familial, autosomal dominant)	Familial hypercholesterolemia (impaired clearance of LDL)
VLDL re-ceptor	Loss-of-function (familial, autosomal recessive)	Autosomal recessive cerebellar hypoplasia (ataxia, mental retardation)
LRP5	Loss-of-function (familial, autosomal recessive)	Osteoporosis-pseudoglioma-syndrome (reduced bone mass)
	Gain-of-function (familial, autosomal dominant)	High-bone-mass trait (increased osteogenic activity)
LRP6	Missense mutation (familial, autosomal dominant)	Autosomal dominant early coronary artery disease (hyperlipidemia, hypertension, diabetes)
LRP1B	Loss-of-function (sporadic)	Esophageal squamous cell carcinoma, nonsmall-cell lung cancer
LRP2/ megalin	Loss-of-function (familial, autosomal recessive)	Donnai-Barrow syndrome (brain malformation, renal tubular deficiency, diaphragmatic hernia)
SorLA	Polymorphisms (sporadic)	Late-onset Alzheimer's disease

surface receptor for ►**reelin**, a secreted guidance factor for newborn neurons that migrate to their proper position in the laminating neocortex and cerebellum. Binding of Reelin to VLDLR and APOER2 initiates an intracellular signaling cascade that involves the cytoplasmic adaptor disabled-1 (►**DAB1**) bound to the receptor tails, and that ultimately leads to reorganization of the cytoskeleton and regulation of cell migration (Fig. 2a) [2]. Receptor gene defects in humans (Table 1) and in animal models result in abnormal layering of neurons in the brain, and in severe neuronal dysfunction (cerebellar dysplasia, ataxia).

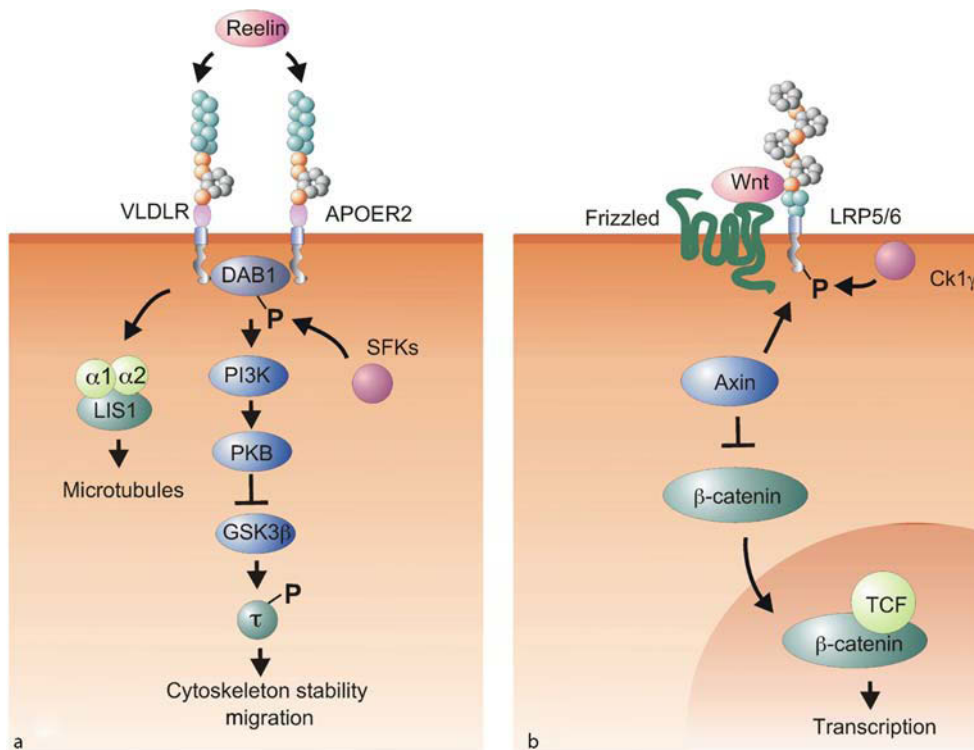
LRP5/6

These receptors are integral components of the ►**wingless** (Wnt) signaling cascade by acting as coreceptors to ►**frizzled**. Simultaneous binding of Wnt ligands to

LRP5/6 and frizzled results in signal transduction through the canonical Wnt signaling pathway (Fig. 2b) [3]. The function of LRP6 seems most relevant for early embryonic patterning events, as loss of receptor function in fruit fly, *Xenopus*, and mouse causes aberrant pattern formation and early embryonic lethality. In contrast, LRP5 activity is mainly required for regulation of bone formation as judged from loss-of-function and gain-of-function syndromes in humans that result in low or high-bone-mass traits, respectively (Table 1).

LRP4

LRP4 is another receptor of the LDL receptor gene family involved in regulation of embryonic patterning, mainly controlling formation of limb structures. Loss of receptor activity in gene targeted mice or spontaneous mutation in bovine cause abnormal limb development and



Low-density Lipoprotein Receptor Gene Family. Figure 2 Signal transduction by members of the LDL receptor gene family. (a) The reelin signaling pathway in migrating neurons is initiated by binding of the guidance factor to its cognate receptors VLDLR and APOER2. Binding induces clustering of DAB1 on the cytoplasmic receptor tails, activating SRC family tyrosine kinases (SFKs) that in turn phosphorylate the adaptor. Phosphorylated DAB1 activates phosphatidylinositol-3-kinase (PI3K) and protein kinase B (PKB). PKB inhibits the activity of glycogen synthase kinase 3β (GSK3β), resulting in reduced phosphorylation of Tau (τ) and stabilization of microtubules. Lissencephaly 1 (LIS1) is another interaction partner of phosphorylated DAB1. It associates with α-subunits to a multimeric protein complex that also regulates microtubule dynamics (modified from [2]). (b) As part of the canonical Wnt signaling pathway, Wnt ligands bind to LRP5/6 and Frizzled resulting in phosphorylation of the LRP5/6 tail by membrane-associated casein kinase 1γ (CK1γ). Phosphorylation recruits scaffold protein Axin to the LRP tail domain, preventing Axin-induced destruction of β-catenin. Stabilized β-catenin enters the nucleus to interact with high-mobility group protein TCF to induce target gene transcription.

polysyndactyly. The molecular mechanisms of LRP4 actions remain unclear at present, but may also involve signaling through embryonic morphogen pathways.

LRP1 and LRP1B

In hepatocytes, LRP1 serves as backup pathway to the LDL receptor in hepatic clearance of ►**chylomicron remnants**, lipoproteins that transport dietary lipids from the intestine to the liver. In vascular smooth muscle cells, LRP1 likely acts as signaling receptor that suppresses the activity of the platelet-derived growth factor (►**PDGF**) receptor. Loss of local LRP1 expression in mouse models causes enhanced activation of the PDGF receptor pathway, resulting in smooth muscle cell proliferation and in marked susceptibility to atherosclerotic lesion formation [4]. LRP1B is also known as LRP-DIT (deleted-in-tumors) because of its original description as a gene frequently inactivated in lung cancer cell lines. A possible role as tumor suppressor gene remains yet to be established. LRP1B may share some functional redundancy with LRP1 as suggested from the close sequence similarity and overlap in expression pattern of both receptors.

LRP2

LRP2 (megalin, gp330) is the largest member of the LDL receptor gene family. Inherited *Lrp2* gene defects are the cause of Donnai-Barrow syndrome, characterized by brain malformation, renal dysfunction, and diaphragmatic hernia. Rather than acting as lipoprotein receptor such as the LDL receptor and LRP1, LRP2 functions as endocytic receptor for cellular uptake of lipophilic vitamins and steroid hormones bound to plasma carrier proteins [1]. Most notably, a role for the receptor in retrieval of filtered 25-OH vitamin D₃ bound to the vitamin D binding protein (►**DBP**) from the glomerular filtrate is appreciated. Renal uptake of vitamin/DBP complexes prevents uncontrolled urinary loss of this essential metabolite and it delivers to renal proximal tubular cells the precursor for conversion into 1,25-(OH)₂ vitamin D₃, the active hormone that controls systemic calcium and bone metabolism. Loss of receptor function results in excessive urinary excretion of vitamin D₃ metabolites, and consequently, in vitamin D deficiency and osteomalacia (softening of the bones).

SorLA

SorLA is a chimeric receptor that harbors protein modules not present in other members of the LDL receptor gene family (Fig. 1). As well as acting as endocytic receptor for extracellular ligands (such as PDGF), sorLA also regulates the intracellular trafficking of target proteins between secretory and endocytic compartments. In particular, the receptor acts as sorting receptor for the amyloid precursor protein (►**APP**), the etiologic agent in ►**Alzheimer's disease**. In neurons, SorLA directs APP into intracellular compartments less

favorable for proteolytic processing, thereby impairing breakdown of APP to the amyloid- β peptide, the principle component of senile plaques (a hallmark of Alzheimer's disease). Polymorphisms in the human *SorLA* gene are among the most important genetic risk factors of late-onset Alzheimer's disease, likely by determining the expression levels of this protective factor in the brain of individuals [5].

Drugs

Currently no drugs directly modulating the LDL receptor family are known. The possible use of drugs targeting the LDL receptor family or downstream signaling proteins may be derived from Table 1.

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Low-density Lipoprotein Receptor-related Proteins

► Low-Density Lipoprotein Receptor Gene Family

Low Molecular Mass GTPases

► Small GTPases

Low Voltage-activated (LVA) Ca²⁺ Channels

► Voltage-dependent Ca²⁺ Channels

LPL

- Lipoprotein Lipase

LPS

- Lipopolysaccharide

LRP

- Low-Density Lipoprotein Receptor Gene Family

LRP5 and LRP6

LRP5 and 6 are closely related genes in humans and mice that are structurally distinct from other *LRP* family members. A single *Drosophila* ortholog exists and is called *arrow*. The proteins are single-pass transmembrane receptors with an extracellular domain comprising four amino-terminal epidermal growth factor (EGF)-like repeats and three low density lipoprotein (LDL) receptor type A repeats. They have a relatively short proline-rich intracellular domain. Genetic evidence in *Drosophila* as well as loss-of-function and overexpression evidence in vertebrates support the conclusion that *arrow* and LRP5/6 are essential coreceptors with the Fz proteins for Wnt/ β -catenin-dependent signaling.

- Wnt Signaling
- Low-Density Lipoprotein Receptor Gene Family

LTD

- Long Term Depression

LTP

- Long Term Potentiation

Luteinizing Hormone

- Contraceptives

Lymphangiogenesis

Lymphangiogenesis is the growth of lymphatic vessels, which is critically controlled by the interaction of VEGF-C and VEGF-D with the receptor VEGF-R3 on lymphatic endothelial cells.

- Angiogenesis and Vascular Morphogenesis

Lymphocytes

Lymphocytes are specialized white blood cells that play a crucial role in an immune response. They can be T lymphocytes, which can directly target and destroy defective cells, or B lymphocytes, which produce antibodies directed against specific antigens. Both T and B lymphocytes produce a variety of cytokines to augment and amplify the immune response.

- Immune Defense

Lymphokines

- Cytokines
- Growth Factors

Lysine Acetylation of Histones

- Histone Acetylation

Lysipressin

- Vasopressin/Oxytocin

Lysolipid Mediators

- Lysophospholipids

Lysophosphatidic Acid

Lysophosphatidic acid (LPA) is the prototype of a group of bioactive *lysophospholipids* that act on specific G-protein-coupled receptors to mediate a wide variety of cellular functions.

- Lysophospholipids

Lysophosphatidylcholine

Synonym

LPC

Definition

Potential signaling phospholipid derived from phosphatidylcholine by phospholipase action. No receptors known.

- Proton-Sensing GPCRs
- Lysophospholipids

Lysophospholipids

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Synonyms

Lysosphingolipids and lysoglycerophospholipids; Lysolipid mediators

Definition

Lysophospholipids are small bioactive lipid molecules characterized by a single carbon chain and a polar head group. Two subgroups can be distinguished: molecules containing the sphingoid base backbone (lysosphingolipids) and molecules containing the glycerol backbone (lysoglycerophospholipids). The lysolipid structure renders these lipids more hydrophilic and versatile than their corresponding phospholipids. Lysophospholipids act as extracellular mediators activating specific ► **G-protein-coupled receptors** (GPCRs), although some of them additionally play a role in intracellular signal transduction. ► **Sphingosine-1-phosphate** (S1P) and ► **lysophosphatidic acid** (LPA) have been characterized in greatest detail so far, and the concept of lysophospholipids as a group of mediators is supported by the high homology between S1P- and LPA-specific GPCRs. Other, less well characterized lysophospholipids are sphingosylphosphorylcholine (SPC), lysophosphatidylcholine (LPC), psychosine (galactosylsphingosine) and glucopsychosine (glucosylsphingosine); the latter two are lacking a phosphate group. Lysophospholipids are auto- or paracrine regulators of cell growth and survival, migration and chemotaxis, cytoskeletal architecture, cell-cell-contacts and adhesion, Ca^{2+} homeostasis and Ca^{2+} -dependent functions. By regulating these cellular functions, lysophospholipids play a role in angiogenesis, lymphocyte trafficking, development of the nervous system, cancer growth and metastasis, inflammation and arteriosclerosis. Relatives of the lysophospholipid family are platelet-activating factor and the ► **endocannabinoids**, arachidonyl ethanolamide and 2-arachidonyl glycerol.

Basic Characteristics

Metabolism and Occurrence

S1P is formed from sphingosine by ► **sphingosine kinases** (SphKs). Degradation of S1P occurs either reversibly by lipid phosphate phosphohydrolases (LPPs) and S1P phosphatases (SPPs), or irreversibly by S1P lyase (SPL) (Fig. 1). The localization of S1P production is highly important since S1P plays a role both as extracellular mediator and as intracellular



Lysophospholipids, Figure 1 Metabolism of S1P (right panel) and LPA (left panel). S1P is formed by sphingosine kinases (SphKs) and degraded by S1P lyase (SPL), S1P phosphatases (SPPs) and non-specific lipid phosphate phosphatases (LPPs). S1P is in equilibrium with sphingosine (SPH) and ceramide (Cer). Shown is also the sphingomyelin cycle which contributes to regulation of ceramide levels. SphK1 occurs cytosolic as well as membrane-bound and can translocate upon cellular stimulation to the plasma membrane or to intracellular sites. SphK2 is a cytosolic enzyme and has not been found at the plasma membrane. Both SphKs have been observed in the nucleus. S1P, depending on the localization where it is generated, can act on so far unknown intracellular target sites, or it can be excreted, for example by the ATP-binding cassette transporter, ABCC1 (8), and act on S1P-GPCRs. LPPs are integral membrane proteins with their catalytic activity directed to the extracellular space or to the luminal side of organelles, regulating extra- and intracellular levels of LPA and S1P, respectively. SPPs and SPL are endoplasmic reticulum proteins with their catalytic sites directed towards the lumen of endoplasmic reticulum and the cytosol, respectively. Both contribute to regulation of intra- and extracellular S1P levels. A major source of extracellular LPA is cleavage of lysophospholipids, predominantly lysophosphatidylcholine (LPC), by a lysophospholipase D named autotaxin (ATX). LPA can furthermore be generated by deacylation of phosphatidic acid (PA), catalysed by phospholipases A₁ or A₂. For this, phosphatidic acid has to be transferred to the outer membrane leaflet as it is the case in shed microvesicles. Recently, a mitochondrial acylglycerol kinase was identified that phosphorylates monoacylglycerol (MAG) and diacylglycerol (DAG), thereby producing LPA and PA generation, respectively. LPA is not only a GPCR agonist, but also activates the transcription factor, peroxisome proliferator-activated receptor-γ (PPARγ). Degradation of LPA occurs by dephosphorylation, catalysed by LPPs, or by acylation, catalysed by LPA acyl transferases (LPAAT).

second messenger (Fig. 1). Intracellular formation of S1P by SphKs is regulated by many diverse stimuli, among them GPCR agonists, ►growth factors, ►cytokines or depolarization. Some stimuli induce a translocation of SphK1 from the cytosol to the plasma membrane, which appears to enable S1P secretion and so-called “inside-out signalling”. Many cell types including mast cells and tumour cells can release S1P. ATP-binding cassette (ABC) transporters have shown to be involved in this process. S1P is furthermore stored in platelets and released upon platelet activation. Accordingly, it has been found in serum in higher concentrations ($\sim 0.5\text{--}0.8\text{ }\mu\text{M}$) than in plasma ($\sim 0.2\text{--}0.4\text{ }\mu\text{M}$). Plasma S1P is bound to albumin and ►lipoproteins, mainly high-density lipoproteins (HDL). Tissue S1P levels are significantly lower, and thus there is a plasma/tissue S1P gradient.

LPA, i.e. monoacyl-glycerol-3-phosphate, can be formed and degraded by multiple metabolic pathways (Fig. 1). Depending on the precursor molecule and respective pathway, the fatty acid chain in LPA differs in length, degree of saturation and position (sn-1 or sn-2), which has an influence on biological activity. LPA

analogs with ether-bound alkyl or alkenyl chains are quantitatively less abundant than those with ester-bound acyl chains. Extracellular LPA is generated to a large part by a lysophospholipase D named ►autotaxin. Autotaxin is an extracellular enzyme that acts as autocrine motility factor of tumour cells. LPA production by autotaxin is essential for embryonic development in mice and apparently contributes most of LPA in plasma. Alternatively, LPA can be produced from surface-exposed phosphatidic acid by phosphatidic acid-selective PLA₁ or secretory type-II PLA₂. Like S1P, LPA occurs in plasma ($\sim 200\text{ nM}$), is formed during coagulation, and present in serum in micromolar concentrations. Differences in the fatty acid composition of plasma- and serum-LPA suggest that the sources of these LPA pools were different. LPA is produced by many cell types, e.g. fibroblasts, adipocytes and tumour cells.

Lysophospholipid Receptors

An overview of lysophospholipid GPCR is presented in Table 1. Presently best characterized are the receptors of the endothelial differentiation gene (EDG)

Lysophospholipids. Table 1 G-protein-coupled lysophospholipid receptors

+Receptor	Endogenous ligands	Main tissue distribution	Signalling	Main biological effects
S1P ₁	S1P (nM) SPC (μM)	Ubiquitous	G _{i/o}	Migration $\uparrow$, proliferation, survival, cell–cell-contacts, angiogenesis, lymphocyte trafficking
S1P ₂		Ubiquitous	G _{i/o} , G _q , G _{12/13}	Migration $\downarrow$, vascular development, differentiation of vascular smooth muscle cells
S1P ₃		Ubiquitous	G _{i/o} , G _q , G _{12/13}	Heart rate $\downarrow$, vascular development, NO-dependent vasorelaxation
S1P ₄		Lymphoid and haematopoietic tissue	G _{i/o} , G _{12/13}	Proliferation and cytokine secretion in T-cells $\downarrow$
S1P ₅		Brain, white matter tracts	G _{i/o} , G _{12/13}	Proliferation, cell rounding
LPA ₁	LPA	Ubiquitous	G _{i/o} , G _q , G _{12/13}	Proliferation, survival, neurite retraction, brain development
LPA ₂		Ubiquitous	G _{i/o} , G _q , G _{12/13}	Proliferation, survival
LPA ₃		Ubiquitous	G _{i/o} , G _q	Implantation and embryo spacing in mice

Receptors	Activators	Main biological effects
P2Y ₉ /GPR23 (LPA ₄)	LPA	
GPR63 (LPA ₅)		Stress fibre formation, neurite retraction
GPR3, GPR6, GPR12	High constitutive activity S1P or SPC	Meiotic arrest of rodent oocytes, increase in synaptic contacts in rat cortical neurons
OGR1	SPC	Proton
GPR4	SPC, LPC	
G2A	LPC, SPC	
TDAG8	Psychosine	

family, S1P₁₋₅ and LPA₁₋₃. They had originally been named after EDG-1, now S1P₁, which was detected in 1990 as an orphan GPCR upregulated during the differentiation of endothelial cells. According to an IUPHAR committee, these receptors are now named after the primary natural ligand and numbered in order of identification. S1P₁₋₃ and LPA₁₋₃ are widely expressed and often found co-expressed within a single cell type. S1P₄ is predominantly expressed in lymphatic and haematopoietic tissues and S1P₅ in brain white matter and skin. The EDG family receptors couple via G_{i/o}, G_{q/11} and G_{12/13} proteins to inhibition of ►adenylyl cyclase, stimulation of phospholipase C and increase in [Ca²⁺]_i, stimulation or inhibition of ►mitogen-activated protein kinases (ERK, JNK and p38) and cell growth, activation of Akt and survival, activation or inhibition of Rho and Rac and rearrangement of the cytoskeleton, and finally stimulation or inhibition of cell migration (for details, see Biological Actions).

Much less is known about other lysophospholipid receptors. LPA₄ and LPA₅ couple via G_s to activation of adenylyl cyclase. GPR3, GPR6 and GPR12 have a high constitutive activity, thereby stimulating adenylyl cyclase, but appear to be further activated by S1P and/or SPC. GPR6 and GPR12 are strongly expressed in mouse brain, and SPC increased synaptic contacts in GPR12-expressing embryonic cortical neurons. GPR3 and GPR12 are expressed in mouse oocytes and mediate a signal that maintains the oocytes in meiotic arrest, a process that is dependent on high cAMP levels. A role for lipid ligands in meiotic arrest is likely

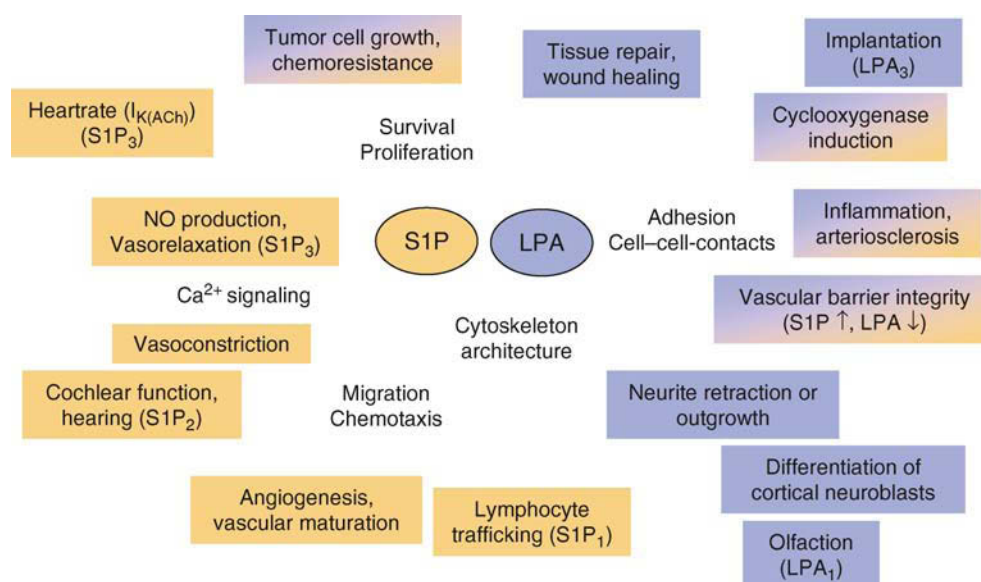
since spontaneous in vitro maturation of rodent oocytes was delayed by SPC and S1P.

Another group of putative lysophospholipid GPCR is highly controversial. OGR1, GPR4, G2A and TDAG8 form a cluster of homologous GPCRs that are candidate high-affinity receptors for SPC, LPC and psychosine (Table 1). SPC and LPC are the phosphocholine-containing analogs of S1P and LPA, respectively. Both lipids are normal constituents of plasma and serum. SPC acts also as a low-affinity or partial agonist at S1P₁₋₅. Contradictory studies have shown that the receptors of the OGR1 cluster can be regulated by lysophospholipids and/or by protons. Recent studies revealed that OGR1 was strongly induced during differentiation of bone marrow mononuclear cells into osteoclasts, and knockdown of OGR1 attenuated osteoclastogenesis. Endogenous GPR4 was required for SPC-stimulated migration of endothelial cells and endothelial tube formation. G2A and TDAG8 appear to play a functional role in migration and apoptosis of immune cells.

Finally, it has to be mentioned that LPA also has an intracellular target site, which is the nuclear transcription factor, peroxisome proliferator-activated receptor-γ (PPARγ). LPA competes for thiazolidinedione binding and activates PPARγ-dependent gene transcription. Thereby, LPA induced neointima formation in a rat carotid artery model.

Biological Actions

Cellular responses to S1P and LPA can be classified as growth-related (stimulation of cell proliferation and



Lysophospholipids. Figure 2 Functional roles of S1P and LPA.

survival, protection from apoptosis), cytoskeleton-dependent (shape change, migration, adhesion, chemotaxis) and Ca^{2+} -dependent (contraction, secretion). Some cellular responses are regulated in an opposite manner by different receptors. For example, the S1P_1 receptor mediates cell migration and chemotaxis, thereby regulating for example angiogenesis and lymphocyte trafficking. Growth factors such as platelet-derived growth factor need the S1P_1 receptor for signalling migration. On the contrary, this must be "and inhibits migration". S1P_2 promotes cellular stress fibre formation and inhibits migration. Furthermore, the S1P_1 receptor does not increase $[\text{Ca}^{2+}]_i$ but rather inhibits $[\text{Ca}^{2+}]_i$ increases by other agonists, while S1P_2 and S1P_3 mediate $[\text{Ca}^{2+}]_i$ increases in response to S1P .

S1P is an important mediator in the cardiovascular system (Fig. 2). S1P stimulates proliferation, survival and migration of endothelial cells and induces formation of adherens junction assembly, decrease in vascular permeability and differentiation of endothelial cells into capillary-like networks. Expression of the S1P_1 receptor within endothelial cells is required for vasculogenesis. Mice lacking this receptor die during embryonic development from haemorrhages caused by a failure of pericytes to migrate around newly formed capillaries. S1P and LPA induce smooth muscle cell contraction and can cause vasoconstriction. S1P , via endothelial S1P_3 receptors, can also cause NO-dependent vasorelaxation. A lack of the S1P_2 receptor results in profound deafness in mice, apparently because the cochlear spiral artery does not appropriately contract in the absence of S1P_2 . The S1P_3 receptor mediates $\text{I}_{\text{K}(\text{ACh})}$ activation in atrial myocytes, leading to bradycardia. However, systemic cardiovascular parameters such as blood pressure or heart rate are marginally affected by intravenous S1P , consistent with a role of S1P as a local rather than systemic mediator. The importance of LPA in vascular development is illustrated by the phenotype of autotaxin deficiency, which is characterized by vascular defects early in development. LPA promotes surface expression of leukocyte adhesion molecules in endothelial cells. In contrast to S1P , LPA enhances vascular permeability. Of the two platelet-derived lysophospholipids, LPA but not S1P stimulates platelet aggregation. S1P , LPA and other lysolipids have been detected in lipoproteins and play a role in atherosclerosis. The major part of lipoprotein-bound S1P is associated with HDL, and many of HDL's beneficial vascular effects are mediated by S1P and S1P -GPCR. In contrast, LPA is produced during mild oxidation of low-density lipoproteins (LDL) and accumulates in atherosclerotic plaques where it acts proinflammatory and promotes thrombus formation.

The S1P_1 receptor and the gradient between lymphatic tissue (low S1P levels) and plasma (high S1P levels) are essential for lymphocyte trafficking. Deletion of S1P_1 , as well as pharmacological

downregulation of S1P_1 (see Drugs), inhibit lymphocyte emigration from lymphatic tissues. Inhibition of S1P lyase leads to elevated S1P tissue levels and also to lymphopenia. It appears likely that the S1P_1 receptor on the lymphocyte surface senses the S1P gradient and mediates lymphocyte trafficking.

LPA receptors play an important role in the nervous system (Fig. 2). The LPA_1 receptor was originally cloned from cerebral cortical neuroblasts and is highly expressed in the neurogenic ventricular zone of the embryonic cerebral cortex. In the adult nervous system, it is predominantly found in myelinating cells, i.e. oligodendrocytes and Schwann cells. Schwann cell morphology, adhesion and survival are regulated by LPA. LPA_1 and LPA_2 receptors mediate neuronal cell rounding and neurite retraction, while LPA_3 appears to stimulate neurite outgrowth. Deletion of the LPA_1 receptor in mice caused ~50% neonatal lethality. The surviving pups had a reduced size, craniofacial dysmorphism and impaired suckling behaviour. The defect in suckling behaviour caused malnutrition of the pups, leading to growth retardation and death, and was attributed to a defective olfaction. Deletion of LPA_2 in mice did not lead to obvious defects, while LPA_3 deficiency was accompanied by ~50% reduced litter sizes. This was caused by downregulation of cyclooxygenase-2 (►cyclooxygenases) in LPA_3 -deficient uteri during preimplantation, which delayed implantation and disturbed embryo spacing. The mice that were born were grossly normal.

Both S1P and LPA stimulate proliferation and migration of fibroblasts, while they inhibit proliferation and induce migration and differentiation of keratinocytes. LPA promotes wound healing *in vivo*. Thus, S1P and LPA interact in tissue repair. Many other tissues are responsive to S1P or LPA, including the respiratory tract (contraction of airway smooth muscle cells and cytokine release by S1P), kidney (diuresis by S1P), adipose tissue (preadipocyte proliferation by LPA), and others. Finally, S1P and LPA promote tumour cell growth, chemoresistance and metastasis (Fig. 2). The two mediators are produced by several tumour cells and occur for example in malignant ascites. The antiapoptotic action of S1P and LPA may protect the cells from chemotherapy or radiation. Furthermore, S1P and LPA stimulate tumour cell motility and invasiveness, although it has to be noted that motility of certain cancer cells (e.g. melanoma cells) is inhibited by S1P . S1P might also play a role in tumour angiogenesis. Its importance for tumour progression is further illustrated by the antitumour activity of SphK inhibitors and an anti- S1P antibody (see Drugs).

In summary, the lysophospholipids are local mediators that regulate development, tissue regeneration and homeostasis, but also play a role in inflammation, arteriosclerosis and cancer.

Drugs

In the field of S1P pharmacology, there is currently a novel immunosuppressive (►[immunosuppressive agents](#)) in clinical development for multiple sclerosis. FTY720 is a prodrug that, after being phosphorylated, acts as an agonist with low nanomolar affinity on S1P₁, S1P₃, S1P₄ and S1P₅, but not on S1P₂ receptors. SphK2 is required for effective phosphorylation of FTY720 *in vivo*. The immunosuppressive action of FTY720 is caused basically by activation and subsequent internalization of the S1P₁ receptor, which needs to be expressed on the lymphocyte surface to enable sensing of the plasma/tissue S1P gradient and to promote emigration of lymphocytes from lymphatic tissues. Since FTY720 does not generally impair T- and B-cell proliferation and functions, it presents a novel mode of immunosuppressive action, which might be useful in multiple sclerosis as well as transplantation or autoimmune diabetes. Activation of S1P receptor subtypes other than S1P₁ causes further, desired and undesired effects. For example, the bradycardia that occurs during the first days of FTY720 treatment is mediated by activation of S1P₃. Animal models show further effects of FTY720: it reduced the damage caused by ischemia-reperfusion in liver and kidney, inhibited angiogenesis, impeded tumour growth in a mouse model of melanoma, induced endothelial NO synthase activation and vasodilatation (via S1P₃) and reduced vascular permeability *in vivo*.

Other compounds acting on S1P-GPCR are only in the experimental stage. Several specific S1P₁ agonists have been described. SEW2871, that has been identified by high-throughput screening and lacks a phosphate group, induces lymphopenia in mice (in agreement with the role of S1P₁ in lymphocyte trafficking) but no bradycardia (in agreement with a lack of an effect on S1P₃). KRP-203 needs to be phosphorylated, like FTY720, but then activates specifically S1P₁ and produces effects similar to those of SEW2871. Interestingly, the S1P₁ receptor has a high constitutive activity which was reduced by an inverse agonist for S1P₁ (SB649146). A recently described S1P₁ antagonist did not induce lymphopenia but reversed immunosuppression induced by S1P₁ agonist, and furthermore enhanced capillary leakage. A putative S1P₃ receptor antagonist, BML-241, was identified by searching a three-dimensional compound database with a pharmacophore model of S1P, however, BML-241 has appears to have other, GPCR-independent effects. A specific S1P₂ receptor antagonist, JTE-013, has widely been used to analyse S1P₂ signalling pathways. This compound prevented for example S1P₂-mediated inhibition of cell migration and contraction of smooth muscle cells. Recently, a series of aryl amide compounds was presented which were more or less receptor subtype-selective. The lead compound, VPC23019, was a competitive antagonist at S1P₁ and S1P₃. K_i values for

VPC23019 in radioligand binding assays at S1P₁ and S1P₃ were in the low nanomolar range, and VPC23019 inhibited S1P-induced migration and Ca²⁺ mobilization. Small structural changes converted the molecule into an agonist. Furthermore, VPC23019 as all compounds of this series behaved as agonist at S1P₄ and S1P₅, but had no activity at S1P₂. Recently, an anti-S1P antibody was described. This antibody retarded the proliferation of various transplanted mouse tumours. Furthermore, it reduced tumour-associated angiogenesis and the ability of S1P to protect tumour cells from apoptosis. An antitumour activity was also observed with SphK inhibitors.

LPA-GPCR pharmacology is at the experimental stage. Dioctylglycerol pyrophosphate (DGPP 8:0) acts as a competitive antagonist preferentially at human LPA₃ receptors and inhibits at higher concentrations LPA₁, but not LPA₂. DGPP 8:0 specifically inhibited LPA-induced platelet activation. Furthermore, it blocked platelet activation by mildly oxidized low density lipoproteins and by homogenates of lipid-rich core isolated from soft arteriosclerotic plaques, illustrating the importance of LPA as a component of arteriosclerotic plaques. Fatty alcohol phosphates (FAP) with carbon chain lengths between 10 and 14 activate LPA₂ and inhibit LPA₃ in a competitive manner. Another LPA-GPCR antagonist is Ki16425, which was identified by high-throughput screening of 150,000 compounds for inhibition of LPA-induced [Ca²⁺]_i increases. This compound inhibited LPA₁ and LPA₃ at slightly lower concentrations than LPA₂, but was highly specific for LPA-GPCR. A similar preference for LPA₁ and LPA₃ was observed with VPC12249. O-methylphosphothionate (OMPT), a LPA₃-selective agonist, aggravated ischemia-reperfusion injury in mouse kidney, while the LPA_{1/3} antagonist, VPC12249, reduced the kidney damage. Since most compounds that interact with LPA-GPCRs, except Ki16425, have a phosphate group that can be subject to dephosphorylation, a novel strategy focuses on metabolically stabilized LPA analogues such as phosphonates, phosphorothioates, phosphonothioates and fluorophosphonates. These compounds affect LPA-GPCRs as well as LPA metabolizing enzymes.

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Lysosphingolipids and Lysoglycerophospholipids

► Lysophospholipids

M-Channels

The M-channels (M for *muscarine*) are expressed in the peripheral sympathetic neurons and CNS. In the absence of acetylcholine, the M-channel opens at resting membrane potential and dampens neuronal responsiveness to synaptic inputs. Acetylcholine inhibits M-channel activity by activation of M1 receptor.

► K^+ Channels

Macula Densa

The macula densa is a dense aggregation of cells in the distal tubule of nephrons facing the glomerular tuft of capillaries. These cells sense the salt content of the distal tubular fluid and adjust glomerular perfusion and renin secretion accordingly.

► Renin–Angiotensin–Aldosterone System
► Diuretics

Macrolides

Macrolides are a group of antibiotics, produced in nature by many actinomycetes strains, that are composed of a 12- to 16-membered lactone ring, to which one or more sugar substituents is attached. They target the peptidyl transferase center on the 50S ribosomal subunit and function primarily by interfering with movement of the nascent peptide away from the active site and into the exit tunnel.

► Ribosomal Protein Synthesis Inhibitors

Major Histocompatibility Complex (MHC)

Group of transmembrane proteins engaged in the presentation of small peptide fragments to T-cells. Two classes of Major histocompatibility complex (MHC) molecules exist both of which are encoded by a highly polymorphic gene cluster. MHC class I and class II proteins present peptide fragments to $CD8^+$ and $CD4^+$ T-cells, respectively. The human MHC is also known as “HLA,” the murine MHC as “H-2” complex.

► Immune Defence

Macrophage

White blood cell preferentially located near potential entry sites for microbial pathogens and specialized for the uptake of particulate material by phagocytosis. Most macrophages originate from peripheral blood monocytes and are able to leave the circulation following stimulation by chemotactic agents.

► Interferons
► Immune Defense

Malaria

Malaria remains a major public health problem in many parts of the world, including Southeast Asia, sub-Saharan Africa and Latin America where an estimated 300–500 million people are infected. 1–3 million die of malaria every year. The etiologic agents of malaria are protozoan parasites of the genus *Plasmodium*. Of the four pathogens that can cause malaria in humans (*Plasmodium falciparum*, *P. vivax*, *P. ovale*,

P. malariae), *P. falciparum* is responsible for the most severe form. At particular risk of developing severe malaria-associated pathology are the non-immune, including tourists and, in endemic areas, children and pregnant women during first pregnancy.

Malarial parasites feature a complex life cycle alternating between vertebrate and invertebrate hosts. The life cycle begins with the bite of an infected *Anopheles* mosquito and the injection of sporozoites into the blood stream. Sporozoites enter the liver and infect hepatocytes where they replicate and produce merozoites. Upon rupture of an infected hepatocyte, merozoites are released. The merozoites infect erythrocytes to establish the erythrocytic developmental cycle. The clinical symptoms of malaria are largely associated with the intraerythrocytic stages and include intermittent fever followed by shaking chills, headache, muscle pain, nausea, vomiting and diarrhoea. Infections with *P. falciparum* can cause life-threatening complications including cerebral malaria and multi-organ failure.

► Antiprotozoal Drugs

Malignant Hyperthermia (MH)

Malignant hyperthermia (MH) is an autosomal-dominant pharmacogenetic disorder that is triggered by exposure to inhalation of general anesthetics, such as halothane. In susceptible individuals, these drugs can induce tachycardia, a greatly increased body metabolism, muscle contracture and an elevated body temperature (above 40°C) with a rapid rate of increase. Many cases of MH are linked to a gene for type 1 ryanodine receptor (RyR1).

► Ryanodine Receptor

Malignant Neoplasm

► Cancer, Molecular Mechanisms of Therapy

Malignant Tumor

► Cancer, Molecular Mechanisms of Therapy

Mannose 6-Phosphate Receptor

The mannose 6-phosphate receptor is the cargo/coat-receptor for trans-Golgi network (TGN)-derived clathrin vesicles. The receptor recognizes the mannose 6-phosphate tag of lysosomal hydrolases on the luminal side and the adaptor-1 complex of clathrin on the cytoplasmic face.

► Intracellular Transport

MAO

► Monoamine Oxidase

MAP

MAP is the acronym for both, ► Microtubule Associated Protein and ► Mitogen Activated Protein.

MAP Kinase Cascades

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Synonyms

Serine/threonine protein kinase phosphorelay modules

Definition

Mitogen activated protein ► kinase (MAPK) cascades are three kinase modules activated by ► phosphorylation. The three kinase modules are composed of a MAPK, a MAPKK, and a MAPKKK. There are multiple members of each component of the MAPK cascade that are conserved from yeast to human. Activation of selective MAPK modules by specific stimuli regulates cell functions such as gene expression, adhesion, migration, differentiation, and apoptosis.

Basic Characteristics

Characteristics of MAPK Cascades

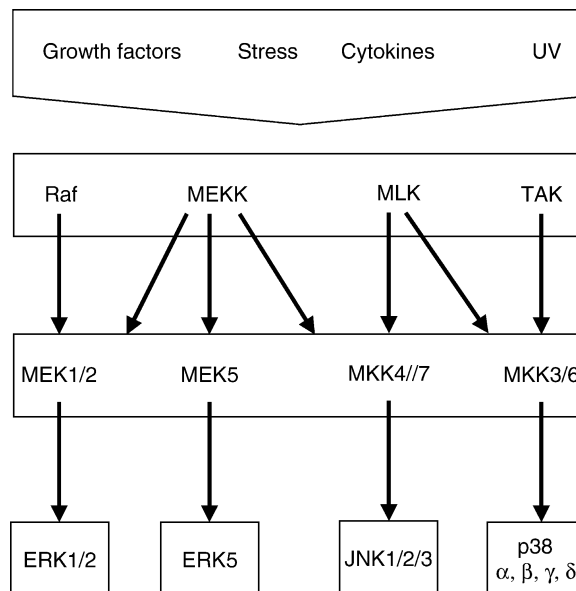
MAPK cascades are signaling modules that serve as important mediators of signal transduction from the cell surface to the nucleus [1, 2]. MAPK modules are activated by diverse stimuli including growth factors, hormones, ►cytokines, bacterial products such as lipopolysaccharide (LPS), and stresses to the cell including gamma and ultraviolet irradiation, heat and cold shock, hyperosmolarity, and oxidative stress. Activation of MAPK signaling by these stimuli contributes to the regulation of many different cell functions including growth, differentiation, survival, ►apoptosis, cytokine production, migration, and adhesion [2].

MAPK cascades are composed of three cytoplasmic kinases, the MAPKKK, MAPKK, and MAPK, that are regulated by phosphorylation (Fig. 1) [1, 2]. The MAPKKK, also called MEKK for MEK kinase, is a serine/threonine kinase. Selective activation of MAPKKKs by upstream cellular stimuli results in the phosphorylation of MAPKK, also called MEK for MAP/ERK kinase by the MAPKKK. MAPKKK members are structurally diverse and are differentially regulated by specific upstream stimuli. The MAPKK is phosphorylated by the MAPKKK on two specific serine/threonine residues in its activation loop. The MAPKK family members are dual specificity kinases capable of phosphorylating critical threonine and tyrosine residues in the activation loop of the MAPKs. MAPKKs have the fewest members in the MAPK signaling module. MAPKs are a family of serine/threonine kinases that upon activation by their respective MAPKKs, are capable of phosphorylating cytoplasmic substrates as well as

translocating to the nucleus where they phosphorylate transcription factors and thus regulate gene expression. MAPKs are divided into four main subfamilies including the extracellular signal-regulated kinases (ERKs) 1 and 2, ERK5, Jun amino-terminal kinases (JNKs), and p38 kinase members. This review will focus on four of the best characterized MAPK cascades including ERK1/2, ERK5, JNK, and p38 kinase.

Characteristics of the ERK1/2 Pathway

Extracellular signal-regulated kinase (ERK) 1 and ERK 2, also called p44 MAPK and p42 MAPK respectively, are nearly 85% identical. ERK1 and 2 are involved in the regulation of cell growth, differentiation, survival, and cell cycle progression. While ERK1 and 2 are ubiquitously expressed in mammalian tissues, the majority of their functions and biological properties have been characterized in the context of fibroblasts [2]. Similar to other MAPKs, ERK1 and ERK 2 are terminal components of a three kinase signaling module. Extracellular stimuli (such as growth factors, cytokines and serum) or events (such as cell adhesion) promote the activation of Raf kinases (MAPKKKs), which proceed to phosphorylate MAPK/ERK kinase (MEK) 1 and MEK2 (MAPKKs). Activation of ERK1/2 is accomplished by the MEK1- or MEK2-mediated dual phosphorylation of threonine and tyrosine of the conserved threonine–glutamate–tyrosine (TEY) motif present in the catalytic domains of ERK1/2 [1]. Once activated, ERK1/2 phosphorylate cytosolic substrates, including ribosomal S6 kinase (p90^{rsk}), phospholipase A₂ (PLA₂), and microtubule associated proteins (MAPs). Additionally, activated ERK1 and ERK2 are capable of translocating to the



MAP Kinase Cascades. Figure 1 Organization of MAPK cascades. See text for details.

nucleus where they phosphorylate a number of transcription factors (Elk1, Ets1, Sap1a, and c-Myc) and, thereby, increase their transcriptional activities [2]. Downregulation of ERK1/2 is achieved chiefly through the dephosphorylation of their TEY motifs by a family of MAPK ►phosphatases (MKPs) [1].

The biological functions of ERK1 and ERK2 have been further defined by mouse models in which the genes coding for these proteins have been knocked out. ERK1 knockout mice appear to be grossly phenotypically normal and fertile but have defects in thymocyte proliferation and maturation. However, ERK2 knockouts are not viable and die early in embryogenesis (day E7.5) as a result of defects in placental development. Raf-1, an upstream activator of ERK1/2, has also been knocked out in mice and displays similar placental defects leading to embryonic mortality. Knockouts of other upstream activators of ERK1/2, including the MAPKKK B-Raf and the MAPKK MEK1, are also embryonic lethal and have vascular and angiogenic defects [2]. Thus, from these studies important roles for ERK1/2 in cell proliferation, migration, differentiation, and angiogenesis can be inferred.

Characteristics of the ERK5 Pathway

The MEK5/ERK5 pathway is a more recently identified MAPK signaling module [1, 2]. The MAPK ERK5, also known as Big MAP kinase (BMK1) possesses a TEY activation motif similar to ERK1/2 and is activated by growth factor stimulation, albeit with slightly different kinetics than ERK1/2. Stimulation of cells with epidermal growth factor (EGF) promotes ERK5-dependent cell proliferation and cell-cycle progression. In addition to being responsive to growth factors, ERK5 is also activated by cell stress stimuli including oxidative stress and hypersosmolarity similar to the MAPK members JNK and p38 kinase. Although ERK5 is stimulated by activators common to other MAPK members, ERK5 is activated by a unique upstream MAPKK termed MEK5 that is not utilized by other MAPKs [1, 2]. MEK5 is activated by the MAPKKKs MEKK2 and MEKK3. Additionally, the structure of ERK5 is significantly different from other MAPK members. ERK5 possesses a unique, long C-terminal tail containing a nuclear localization signal and a distinct N-terminal domain that binds MEK5.

ERK5 is an important regulator of serum-induced immediate early gene expression. Active ERK5 has been shown to phosphorylate MEF2c, a member of the MEF2 transcription factor family, resulting in an increase in c-jun transcription, a gene required for cell proliferation. The essential role of MEK5 and ERK5 in blood vessel and cardiovascular development has been demonstrated in mice deficient for either ERK5 or MEK5. Embryos that lack either ERK5 or MEK5 die at embryonic day 10 due to placental, angiogenesis, and cardiovascular defects. Deletion of ERK5 in adult mice

results in the apoptotic death of endothelial cells, resulting in leaky blood vessels and hemorrhage. Thus, ERK5 plays an important biological role in the coordination of cell response to different stimuli and promotes cell growth in the vascular system of the both the developing embryo and the adult.

Characteristics of the JNK Pathway

The MAPKs termed Jun amino-terminal kinases (JNKs) are a subgroup comprised of three known members, JNK1, 2, and 3. This group of kinases is thus named due to their specific phosphorylation of the transcription factor c-Jun, a component of the AP-1 transcription complex. JNK pathway regulation of AP-1 activity is critical for transcriptional control of a number of gene products resulting from a variety of stimuli, ranging from growth factor receptor ligation, cytoskeletal changes, and cellular stresses like osmotic shock and irradiation. Indeed, JNKs were originally identified as stress-activated protein kinases (SAPKs) [1, 2]. Through the regulation of AP-1, JNKs influence the expression of genes as diverse as cytokines, growth factors, inflammatory mediators, and matrix ►metalloproteinases. Thus, JNK signaling is expected to play a key role in cellular survival and disease processes stemming from inflammation and cell death. These pathological conditions include diabetes, stroke, and Parkinson's disease. JNKs 1 and 2 are widely expressed, whereas JNK3 expression is limited to neuronal tissue and cardiac myocytes [2, 3].

JNKs are also classified by the presence of a threonine–proline–tyrosine motif in their activation loop. These residues are phosphorylated by the upstream dual-specificity MAPKKs MKK4 and MKK7 resulting in JNK activation. MKK4/7 are, in turn, phosphorylated by a number of upstream MAPKKKs, including MEKK1/2/3/4, MLKs, ASK1, TAO1 and 2, and TAK1 [1]. Several JNK pathway component knockouts have been produced by ►homologous recombination, and these tools are helping to define the biological function of JNK signaling. These studies have thus far shown JNK1 and 2 as having a role in immune cell function. Simultaneous deletion of both JNK1 and 2 results in death during midgestation due to failure of neural tube closure and additional brain defects. All JNKs seem to regulate cell survival. For example, JNK1/2 double knockout fibroblasts show increased resistance to radiation [1, 3].

Characteristics of the p38 Kinase Pathway

p38 kinases are members of the mitogen-activated protein kinase (MAPK) family of serine/threonine kinases that phosphorylate and regulate both cytoplasmic proteins and transcription factors, thus regulating gene transcription [1]. Several investigators have independently identified p38 kinase during experiments designed to isolate kinases involved in cell responses to cellular stresses. Four isoforms of p38 kinase have been isolated:

α , β , γ , and δ . These isoforms have been classified as p38 kinases based on both the presence of a conserved threonine–glycine–tyrosine (TGY) sequence in their activation loop and a high amino acid identity. For example, p38 α and β are 60% identical to p38 γ and δ , further suggesting p38 kinases as a subfamily of MAPKs [2]. Stimuli including heat and cold shock, osmotic shock, irradiation, LPS, and cytokines have been shown to activate p38. The MAPKKs activated upstream of p38 include MEKK3, MEKK4, ASK1, TAK1, and MLK3. These MAPKKs have been shown to activate the MAPKKs MKK3/MKK6 that phosphorylate p38 kinase on a highly conserved tyrosine and threonine residues in the activation loop. Phosphorylation of p38 kinase results in the nuclear translocation of this kinase where it can phosphorylate and activate transcription factors such as ATF-2 and ELK1. However, not all targets of p38 kinase are nuclear. p38 kinase also phosphorylates cytoplasmic proteins such as tau protein, MAPKAPK2, 3 and 5, and Mnk1/2.

Activation of p38 has been shown to affect multiple cell functions including development, growth, ►apoptosis, spreading, adhesion, cytokine production and cell-cycle progression. Embryos deficient for p38 α die midgestationally by embryonic day 12.5 due to defects in placental angiogenesis, whereas mice lacking p38 β , p38 γ , or p38 δ are viable, suggesting functional redundancy of these p38 isoforms. Gamma irradiation activates MKK6, stimulating p38 γ and blocking G2-M transition. Similarly, UV irradiation activates p38 α and β that may

serve as an early sensor of DNA damage. Finally, stimulation of cells with LPS activates p38 kinase, resulting in the production of the ►cytokines IL-1 and TNF α and leading to increased inflammation. p38 further increases inflammation through the stabilization of mRNAs containing AU repeats such as TNF α and COX2. Disruption of the p38 pathway would therefore be useful clinically in the arrest of the inflammatory response and in the treatment of inflammatory diseases [3].

Drugs

To date, no compounds have been identified that directly and specifically inhibit either ERK1 or ERK2. However, a number of inhibitors have been developed that affect the activities of the kinases upstream of ERK1/2. The most useful compounds for the inhibition of ERK1/2 activity have been the MEK1/2 inhibitors PD98059 and UO126 (Table 1). These compounds have high specificity for MEK1 and MEK2, and at low concentrations, do not cause appreciable inhibition of MAPK pathways other than ERK1 and ERK2. Both PD98059 and UO126 bind to MEK1 and MEK2 at sites distinct from the ATP-binding pocket, but cause allosteric changes in the proteins that render them unable to activate ERK1/2. However, neither of these two inhibitors is suitable for clinical use due to unfavorable pharmacological properties. A second-generation compound, PD184352, has been developed and found to successfully reduce elevated levels of ERK1/2 activity in colon carcinoma cells and reduce tumor growth in mice (Table 1) [3]. PD184352

MAP Kinase Cascades. Table 1 Pharmacological Inhibitors of MAPK Cascades

Kinase	Inhibitors	Mechanism of Action	Biological Consequences
Raf	Bay 439006	ATP-competitive	Reduces MEK1/2 activity and tumor growth.
MEK1/2	PD98059 U0126 PD184352 PD0325901 ARRY-142886	Binds to MEK1/2 at a site distinct from the ATP binding pocket.	Reduces ERK1/2 activity and cancer cell growth.
MEK5	PD98059 U0126 PD184352	Unknown	Reduces ERK5 activity by partial blocking of MEK5.
JNK1/2	SP600125	Binds the ATP binding pocket of JNK.	Blocks LPS-induction of TNF α expression. Blocks TCR mediated apoptosis.
p38 kinase α/β	SB203580 SB202190 VK19911 Vertex 745 Vertex 702 Vertex 850 BIRB-796 SB235699 SCIO-469 SCIO-323	Binds the ATP binding pocket of p38 kinase.	Blocks p38 stimulation of production of pro-inflammatory cytokines.

was withdrawn from clinical trials due to poor efficacy, likely resulting from poor solubility, high metabolic clearance, and poor bioavailability [4]. More recently, PD0325901 and ARRY-142886 have entered clinical trials [4]. In addition to inhibitors of MEK1/2, a Raf-1 inhibitor, Bay439006, has shown promise as an inhibitor of ERK1/2-mediated cancer cell growth and ▶[metastasis](#) and is in Phase II and III trials in patients with many different tumor types including melanoma and colorectal cancer [4].

Selective inhibitors of ERK5 have not been described. However, studies of MEK1/2 inhibitors have detected effects on MEK5, the upstream activator of ERK5. In addition to inhibition of MEK1/2 activation of ERK1/2, both PD98059 and U0126 have been shown to partially reduce EGF and hydrogen peroxide activation of the ERK5 pathway in mammalian cell lines (Table 1). Inhibition of the ERK5 pathway occurs at the level of MEK5, however the precise mechanism is unknown. Similarly, the MEK1/2 inhibitor PD184352 also inhibited EGF-stimulation of the MEK5/ERK5 pathway, but a 10-fold higher concentration of inhibitor was required relative to that required for MEK1/2 inhibition (Table 1). Additionally, low doses (2 μ m) of PD184352 that completely block ERK1/2 activity were shown to prolong activation of ERK5 in response to EGF and hydrogen peroxide. The additional effects of MEK1/2 inhibitors on MEK5/ERK5 signaling alters the interpretation of results related to studies of these inhibitors on ERK1/2 signaling [3]. Although selective inhibitors of MEK5/ERK5 are yet to be identified, the ability of ERK5 signaling to promote cell proliferation makes it a potentially useful clinical target for inhibitors.

The recent development of the JNK-specific inhibitor SP600125 will likely aid efforts to define the biological significance of JNK signaling (Table 1). SP600125 is an anthrapyrazolone that competes with ATP for the nucleotide-binding site in the JNK catalytic domain [3]. Importantly, as JNK activity is necessary for expression of some extracellular proteinases linked to invasiveness, JNK inhibition may represent an avenue by which to affect tumorigenesis, cancer ▶[metastasis](#), and inflammatory immune response. Further, manipulation of JNK activity may potentially allow alleviation of cell death-mediated pathologies, such as ischemic injury and myocardial infarction.

The most extensive development of pharmacological inhibitors of MAPK cascades members has been for p38 (Table 1) [3]. Small-molecule inhibitors have been developed for two p38 isoforms (α and β). Pyridinyl imidazole compounds have been known to block inflammation since the early 1970s. Structural analyses have revealed that p38 kinase inhibitors binds to the ATP-binding pocket of p38 thereby acting as competitive inhibitors. The p38 kinase inhibitor SB202190 is able to bind both the low-activity nonphosphorylated

form and the high activity phosphorylated form of p38 suggesting that the inhibitor is able to disrupt the activation of p38 (Table 1) [3]. Several p38 inhibitors have been in development for the treatment of inflammatory diseases like rheumatoid arthritis, Crohn's disease, and psoriasis [5]. However, Vertex 745 and BIRB-796 were withdrawn due to effects on the central nervous system and liver enzymes, respectively [5]. Second-generation inhibitors like Vertex 702 and Vertex 850 are in Phase II clinical trials. In addition to reducing inflammation, p38 inhibitors like SCIO-469 and second generation inhibitor SCIO-323 are in clinical trials for the reduction of dental pain associated with inflammation [5].

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MAPK

Mitogen activated protein kinase.

▶[MAP Kinase Cascade](#)

Marijuana

Marijuana is the name given to the dried leaves and flower heads of the hemp plant, *Cannabis sativa*, prepared as a smoking mixture.

▶[Cannabinoid System](#)

▶[Endocannabinoid System](#)

Marimastat

► Matrix Metalloprote(in)ases

Mast Cells

Mast cells are connective tissue cells that can release histamine under certain conditions.

► Allergy
► Histaminergic System

Matrix Metalloproteinases

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Synonyms

MMPs

Definition

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases and belong to a larger family of proteases known as the metzincin superfamily. MMPs are a major group of enzymes that regulate cell–matrix composition. They require a zinc ion in their active site for catalytic activity and are critical for maintaining tissue allostasis. MMPs are active at neutral pH and can therefore catalyze the normal turnover of ► **extracellular matrix** (ECM) macromolecules. Members of the MMP family include the “classical” MMPs (extracellular proteolytic enzymes), the membrane-bound MMPs (MT-MMPs), the ADAMs (a disintegrin and metalloproteinase; adamylsins), and the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif).

Basic Characteristics

MMP genes show a highly conserved modular structure. They were first detected in vertebrates (1962), and human beings, but have since been found in invertebrates and plants as well. The MMP family

consists of zinc-dependent proteolytic enzymes, which comprise at least 24 different members so far [1] (Table 1). Although all of them exhibit a broad substrate spectrum, they are divided based on their main substrate into collagenases, gelatinases, stromelysins, matrilysins, metalloelastase, membrane-type MMPs (MT-MMPs), and others. MMPs are secreted as zymogens and become activated by cleavage of their propeptide. Figure 1 (Domain structure) depicts an overview of the domain organization of different MMPs. They have several structural features in common that include a propeptide domain containing the “cysteine switch,” the catalytic zinc-binding domain with the sequence HEXGHXXGXXHS, and a hemopexin-like domain. Although each MMP exhibits substrate specificity toward individual matrix proteins, there is considerable overlapping within the whole family. Collectively they are capable of degrading all kinds of ECM proteins, such as the interstitial and ► **basement membrane** collagens, proteoglycans (e.g., aggrecan, decorin, biglycan, fibromodulin, and versican) as well as accessory ECM proteins such as fibronectin. MMPs can also process a number of bioactive molecules. They are involved in the cleavage of cell surface receptors, the release of apoptotic ligands (such as the ► **FAS ligand**), and ► **chemokine** (in-)activation. They also play a major role on cell proliferation, migration (adhesion/dispersion), differentiation, ► **angiogenesis**, ► **apoptosis**, and host defense.

The enzymatic activity of these potentially harmful enzymes is tightly controlled. Once transcribed into protein, MMPs are expressed as inactive zymogens and require distinct activation processes to convert them into active enzymes. After secretion, MMP-activity is regulated by the noncovalent binding of tissue inhibitors of metalloproteinases (► **TIMPs**) as shown in Fig. 2 for MMP-2 and TIMP-2. Four TIMPs have been identified so far: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. All known MMPs can be inhibited by at least one of the four known TIMPs. Nevertheless, individual differences with regard to bond strength and thus the magnitude of inhibition of a particular MMP do exist.

Drugs

Ample evidence exists on the role of MMPs in normal and pathological processes, including tissue remodeling associated with various physiological and pathological processes such as morphogenesis, embryogenesis, angiogenesis, tissue repair, inflammation, cirrhosis [2], arthritis, cancer, and metastasis. The gelatinases MMP-2 and MMP-9 are thought to be important, e.g., in metastasis and MMP-1 in rheumatoid and osteoarthritis.

MMP inhibitor development constitutes an important branch of research in both academic and industrial settings and advances our knowledge on the structure–function relationship of these enzymes. Targeting

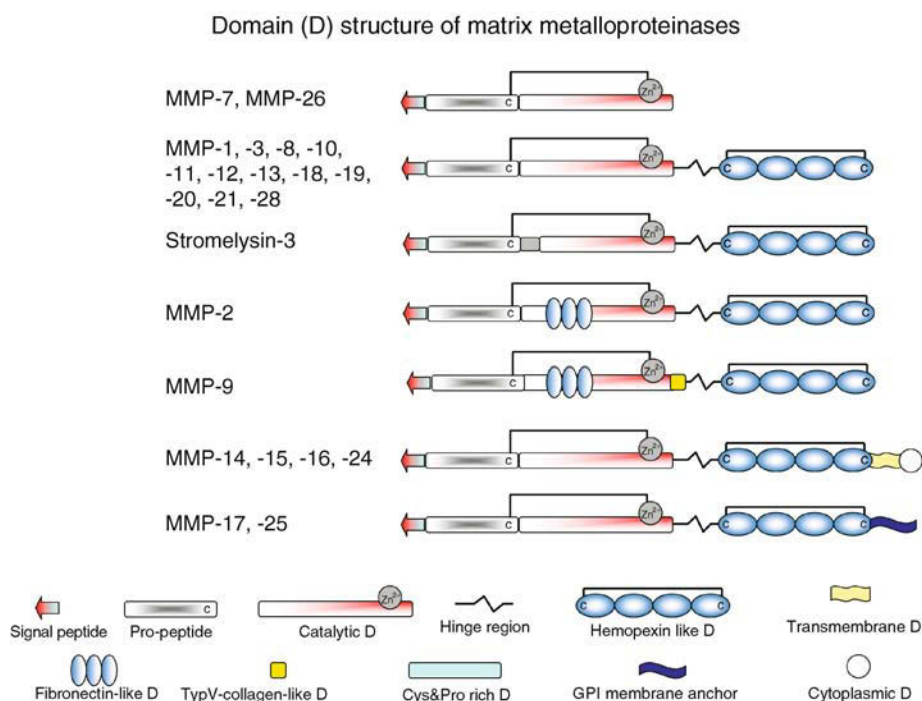
Matrix Metalloproteinases. Table 1 MMPs and their genes known so far

Gene	Name	Location	Comments
<i>MMP-1</i>	Interstitial collagenase	Secreted	One of three collagenases that can degrade the interstitial collagens, types I, II, and III
<i>MMP-2</i>	Gelatinase-A, 72 kDa gelatinase	Secreted	
<i>MMP-3</i>	Stromelysin 1	Secreted	
<i>MMP-7</i>	Matrilysin, PUMP 1	Secreted	Smallest member of MMPs
<i>MMP-8</i>	Neutrophil collagenase	Secreted	
<i>MMP-9</i>	Gelatinase-B, 92 kDa gelatinase	Secreted	MMP-9 plays a regulatory role in angiogenesis not only through proteolytic activity but also through other downstream angiogenic factors
<i>MMP-10</i>	Stromelysin 2	Secreted	
<i>MMP-11</i>	Stromelysin 3	Secreted	MMP-11 shows more similarity to the MT-MMPs, is convertase-activatable and is secreted therefore usually associated to convertase-activatable MMPs.
<i>MMP-12</i>	Macrophage metalloelastase	Secreted	
<i>MMP-13</i>	Collagenase 3	Secreted	
<i>MMP-14</i>	MT1-MMP	Membrane-associated	Type-I transmembrane MMP
<i>MMP-15</i>	MT2-MMP	Membrane-associated	type-I transmembrane MMP
<i>MMP-16</i>	MT3-MMP	Membrane-associated	Type-I transmembrane MMP
<i>MMP-17</i>	MT4-MMP	Membrane-associated	Gglycosyl phosphatidylinositol-attached
<i>MMP-18</i>	Collagenase 4, xcol4, xenopus collagenase	–	No known human orthologue
<i>MMP-19</i>	RASI-1, occasionally referred to as stromelysin-4	–	
<i>MMP-20</i>	Enamelysin	Secreted	
<i>MMP-21</i>	X-MMP	Secreted	
<i>MMP-23A</i>	CA-MMP	Membrane-associated	Type-II transmembrane cysteine array
<i>MMP-23B</i>	–	Membrane-associated	Type-II transmembrane cysteine array
<i>MMP-24</i>	MT5-MMP	Membrane-associated	Type-I transmembrane MMP
<i>MMP-25</i>	MT6-MMP	Membrane-associated	Gglycosyl phosphatidylinositol-attached
<i>MMP-26</i>	Matrilysin-2, endometase	–	
<i>MMP-27</i>	MMP-22, C-MMP	–	
<i>MMP-28</i>	Epilysin	Secreted	Discovered in 2001, given its name due to have been discovered in human keratinocytes. Highly expressed in lung, placenta, salivary glands, heart, uterus, skin

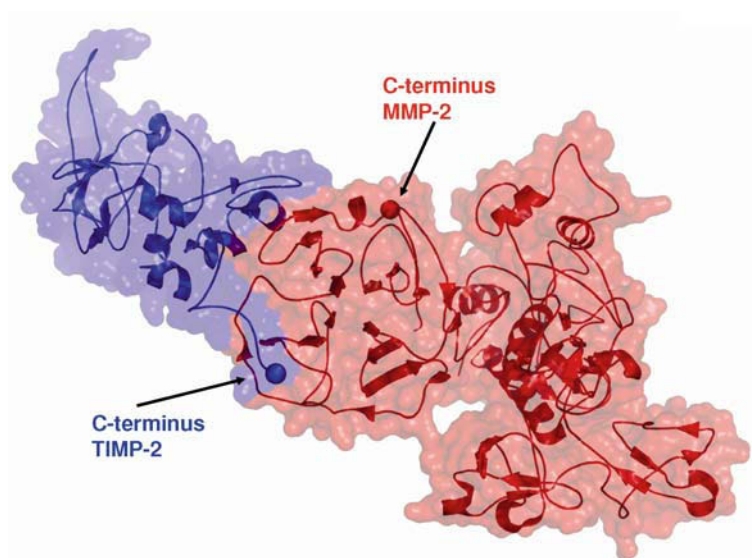
MMPs for therapeutic interventions is complicated by the fact that MMPs are indispensable for normal development and physiology and by their multifunctionality, possible functional redundancy or contradiction, and context-dependent expression and activity. This complexity was revealed by previous efforts to inhibit MMP activity in the treatment of cancer patients that yielded unsatisfactory results. Doxycycline, at subantimicrobial doses, inhibits MMP activity, and has been used in various experimental systems for this purpose. It is used clinically for the treatment of

periodontal disease and is the only MMP inhibitor that is widely available clinically.

A number of rationally designed MMP inhibitors have shown some promise in the treatment of pathologies, which MMPs are suspected to be involved in. However, most of these, such as Marimastat (BB-2516), a broad spectrum MMP inhibitor, or trocade (Ro 32–3555), an MMP-1 selective inhibitor, have performed poorly in clinical trials. The failure of Marimastat was partially responsible for the folding of British Biotech, which developed it. The failure of



Matrix Metalloproteinases. Figure 1 Domain organization of different mammalian MMPs.



Matrix Metalloproteinases. Figure 2 ProMMP-2-TIMP-2 structure (adopted from [4]). TIMP-2 cartoon and transparent surface structure is shown in blue, MMP-2 in red. The C-terminal ends of both molecules are marked as spheres.

these drugs has been largely due to toxicity (particularly musculo-skeletal toxicity in the case of broad spectrum inhibitors) and failure to show expected results (in the case of trocade, promising results in rabbit arthritis models were not replicated in human trials). The reasons behind the largely disappointing clinical results

of MMP inhibitors are still unclear, especially in light of their activity in animal models. New evidence suggests that MMPs may also generate angiogenesis inhibitors [3]. Because of this dual role in tumor tissue, it is important to identify correctly the MMPs involved in function, activity, and origin as a prerequisite to

creating highly selective MMP inhibitors as potential therapeutics.

Development of MMP null mice carrying specific MMP deletions has provided an opportunity to explore the role of MMPs in normal development as well as in such diverse conditions and diseases as skeletal dysplasias, coronary artery and heart disease, arthritis, cancer, and brain disorders.

- Angiogenesis and Vascular Morphogenesis
- Endothelins
- Non-viral Peptidases

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Matrix-assisted Laser Desorption/Ionization Mass Spectrometry

Matrix-assisted laser desorption mass spectrometry (MALDI-MS) is, after electrospray ionization (ESI), the second most commonly used method for ionization of biomolecules in mass spectrometry. Samples are mixed with a UV-absorbing matrix substance and are air-dried on a metal target. Ionization and desorption of intact molecular ions are performed using a UV laser pulse.

- Proteomics

Maturity-Onset Diabetes of the Young (MODY)

MODY is a type of non-insulin-dependent diabetes mellitus caused by rare autosomal-dominant mutations. Presently there are six known forms of the disease which are all due to ineffective insulin production or

release. Mutations are localized in the following genes: hepa-tocyte nuclear factor 4alpha (HNF4alpha, MODY 1); glucokinase (MODY 2); HNF1alpha (MODY 3); insulin promoter factor-1 (MODY 4); HNF1beta (MODY 5); neurogenic differentiation 1 (MODY 6). MODY 2 and MODY 3 are the most common forms. As a rule, in MODY clinical phenotype is similar to mild type 1 diabetes.

- Diabetes Mellitus
- ATP-dependent K⁺ Channels

MCH

- Melanin-concentrating Hormone.

MCR

- Gluco-mineralocorticoid Receptors

MDMA

- 3,4-Methylenedioxymeth-Amphetamine
- Psychostimulants

MDR-ABC Transporters

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Synonyms

Multidrug resistance proteins

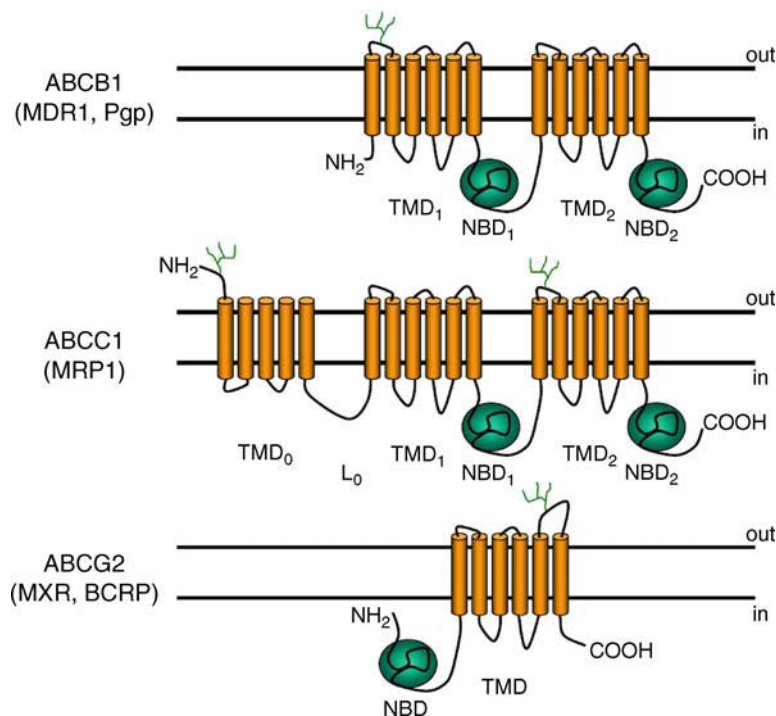
Definition

Multidrug **ABC transporters** are integral membrane proteins that can cause cross-resistance to several cytotoxic agents in human tumors and in cellular model systems. These transporters belong to the so-called ATP-Binding Cassette (ABC) protein family. The human genome encodes 48 ABC proteins that can be grouped into seven subfamilies (ABCA–ABCG). Only three of the 48 ABC transporters, ABCB1 (**P-glycoprotein**, Pgp, MDR1), ABCC1 (**MRP1**), and **ABCG2** (MXR, BCRP) have unambiguously been shown to cause cellular **multidrug resistance** (MDR) [1]. Few other members of the ABC family are also thought to be capable of drug extrusion. Although ABC transporter proteins were discovered and are still referred to as MDR transporters, their major physiological function is to provide general protection against hydrophobic **xenobiotics** [2]. The human (mammalian) multidrug transporters are plasma membrane pumps, exporting compounds from the cytosol to the extracellular space. Transported substrates include cytotoxic drugs, detoxified metabolites, toxins, and carcinogens found in food products, as well as endogenous compounds.

Basic Characteristics Structure

ABC transporters are large integral membrane proteins consisting of transmembrane domains (TMDs) and nucleotide binding domains (NBDs). Secondary structure predictions and membrane topology models indicate that the NBDs are intracellular globular units, whereas in most cases the TMDs are composed of six membrane spanning helices. According to a general consensus, all functionally active ABC transporters contain a minimum of two ABC units and two TMDs. These four elements may be encoded by a single polypeptide chain (“full transporters,” such as the MDR1/Pgp/ABCB1 protein). In contrast, “half transporters,” such as the members of the ABCG family, possess only a single ABC and a single TMD and they form homodimers or heterodimers to generate a functional ABC transporter (Fig. 1). In each case, the tandem repeat of TMDs and ABCs fold into a single transporter with a pseudo-twofold symmetry, in which the transmembrane helices define a “pore” for substrate translocation, and the nucleotide binding cassettes harvest the energy of ATP binding and hydrolysis.

The NBD unit harbors several conserved sequence motifs (Walker A and B, ABC-signature motifs), which



MDR-ABC Transporters. Figure 1 Membrane topology models of the ABCB1 (MDR1/Pgp), the ABCC1 (MRP1), and the ABCG2 (MXR/BCRP) proteins. The models were constructed on the basis of sequence analysis and experimental data. TMD, transmembrane domain; NBD, nucleotide binding domain; L₀, linker region between TMD₀ and TMD₁ in ABCC1.

play a role in harvesting the energy of ATP. Crystallized ABC domains show a unique, L-shaped fold, in which the secondary structure elements form a characteristic assembly only seen in ABC-ATPases. Structural information, together with experimental data has convincingly demonstrated that the two ABC subunits minimally required for a functional ABC transporter cooperate during the transport. The two ABC domains complement each other's active sites, forming two composite catalytic centers per protein.

In all MDR-ABC transporters, the sites interacting with the transported substrates are most probably located within the TMDs. As of today, high resolution structures are not available for mammalian ABC transporters. Therefore, only structural data of bacterial ABC transporters and homology models can serve as a basis of structure predictions for the human MDR-ABC proteins. Recent structural studies of the bacterial multidrug ABC-transporter Sav 1866 indicate a relatively large drug-binding pocket within the transmembrane regions. The transmembrane helices form a cone-shaped chamber, which is lined with hydrophobic side chains, and has a wide opening from the intracellular side. The two ABC domains are in contact with the transmembrane helices thus providing the structural basis of the ATP-coupled transport mechanism.

Mechanism of Action

The MDR ABC proteins are primary **▶active transporters**. As such, they utilize the energy of cellular ATP for the promotion of vectorial, transmembrane movement of drugs or xenobiotics. These ATP hydrolytic enzymes (ATPases) interact with two different types of substrates: the energy donor substrate and the transported substrate. The energy donor substrate is the intracellular Mg-ATP complex, and the chemical energy for the active transport of substrates is provided by the binding and hydrolysis of ATP within the NBD units. In a phenomenological sense, the transported substrates are bound to intracellular (or in some cases probably intramembrane), high affinity "on" sites and are unloaded at extracellular, low affinity "off" sites. While ABC transporters most likely recognize hydrophilic substrates and drug conjugates in the cytosol, hydrophobic substrates that are expected to freely diffuse into the cells are recognized in the context of the plasma membrane, before they reach the cytoplasm ("hydrophobic vacuum cleaner" model).

Anticancer Drug Resistance

Despite recent developments, effectiveness of **▶chemotherapy** is still rather limited for most types of cancer, including tumors of the colon, lung, kidney, pancreas, and liver. Why some cancers respond better than others may be explained by factors relating to the anatomy and physiology of the cancer-ridden organ or

the pharmacokinetics of the drugs used to combat the disease. In addition, tumors may resort to cellular mechanisms, which may prevent the accumulation of cytotoxic drugs in the cancer cells. Of the 48 human ABC transporters, ABCB1 (MDR1/Pgp), several members of the ABCC subfamily (the MRPs) and the **▶ABCG2** protein qualify for the MDR-ABC protein status [3–5]. MDR transporters can export most neutral and cationic hydrophobic compounds, and cancer cells readily co-opt this mechanism as a primary shield against chemotherapy (Fig. 2). This "first-line" defense is reasonably successful, as most of the routinely used agents of the current chemotherapy regimens are MDR transporter substrates.

Although in vitro models clearly show that MDR transporters can protect tumor cells, their relevance in clinical oncology remains controversial. As is the case for most potentially useful cancer biomarkers, no universally embraced guidelines for analytical or clinical validation of MDR transporters exist. Evidence linking ABCB1 Pgp/MDR1 expression with poor clinical outcome is most conclusive for breast cancer, sarcoma, and certain types of leukemia. The relevance of the other MDR transporters in clinical MDR is still unclear. The prognostic implication of ABCC1/▶MRP1 remains controversial and very little is known clinically about ABCG2.

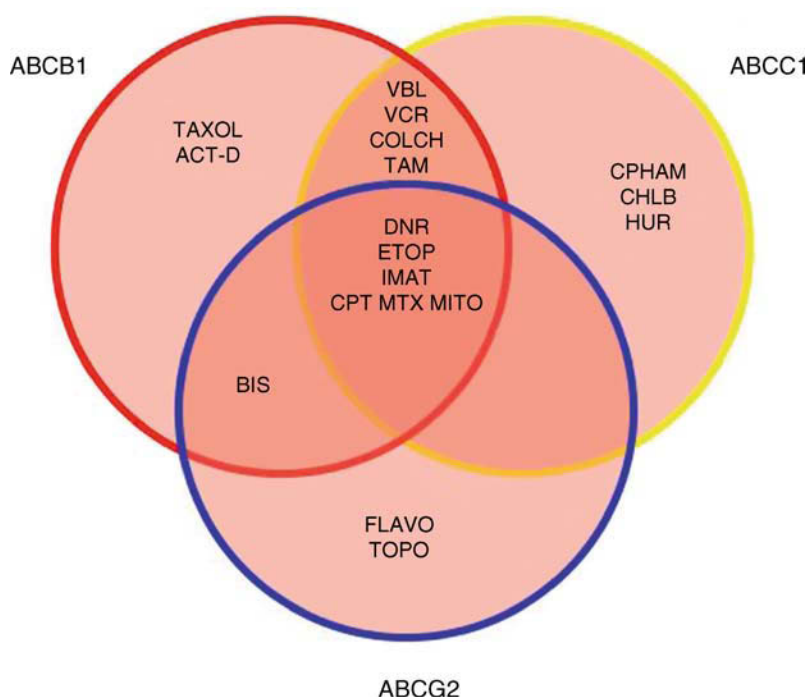
Pharmacological Interventions

Since ABC transporters mediate resistance to a whole array of drugs, they should be an attractive target for the improvement of anticancer therapy. Despite promising in vitro results, successful modulation of clinical MDR through the chemical blockade of drug efflux from cancer cells remains elusive, due to the parallel inhibition of MDR transporters residing in **▶pharmacological barriers**. Modulators are unlikely to improve the therapeutic index of anticancer drugs unless agents that lack significant pharmacokinetic interactions are found.

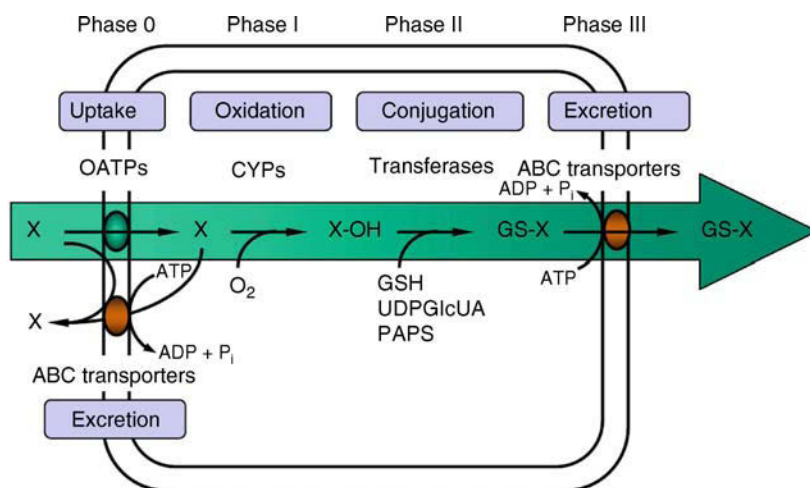
Role of MDR Transporters in Pharmacology

MDR1/Pgp confers resistance to a vast array of clinically and toxicologically relevant compounds, including but certainly not limited to anticancer drugs, HIV-protease inhibitors, antibiotics, antidepressants, antiepileptics, and analgesics. MDR-ABC transporters show high expression in cell types forming different **▶physiological barriers** such as epithelial cells lining the gastrointestinal tract, proximal tubule cells of the kidney, hepatocytes, epithelial cells in the choroid plexus, and capillary endothelial cells in the brain and the testes.

▶Cellular defense mechanisms against toxins (A multistep mechanism for elimination of toxic metabolites and xenobiotics. It involves various transport, oxidation, and conjugation steps.) are usually divided into several steps as it is visualized on Fig. 3. Organic anion transporting proteins (OATPs) are responsible for the cellular uptake of endogenous compounds and



MDR-ABC Transporters. Figure 2 Anticancer agents as substrates of ABCB1, ABCC1, and ABCG2. ACT-D, actinomycin D; VBL, vinblastine; VCR, vincristine; COLCH, colchicin; TAM, tamoxiphen; DNR, daunorubicin; ETOP, etoposide; IMAT, imatinib; CPT, camptothecins; MTX, methotrexate; MITO, mitoxantrone; BIS, bisantrene; FLAVO, flavopiridol; TOPO, topotecan; CPHAM, cyclophosphamide; CHLB, chlorambucil; HUR, hydroxyurea.



MDR-ABC Transporters. Figure 3 Detoxification cellular mechanisms. X, toxic compound; X-OH, oxidized toxic compound; GS-X, conjugated toxic compound; OATP, organic anion transporting proteins; CYPs, cytochromes; GSH, glutathion; UDPGlcUA, Uridine 5'-diphosphoglucuronic acid; PAPS, 3'-phosphoadenylylsulfate.

xenobiotics, whereas MDR-ABC proteins hinder the cellular uptake of these substances (Phase 0). Should toxins enter the cells, they are subject to oxidation (Phase I), and subsequent conjugation (Phase II). As a result of Phase I–II metabolism, toxins become more hydrophilic and are expelled from the cells via

mechanisms that involve certain MDR-ABC transporters (Phase III).

Crucial data regarding the physiological relevance of MDR transporters came from knockout mice studies. Surprisingly, loss of these genes does not result in an obvious phenotype: MDR knockout mice are viable and

fertile, almost indistinguishable from their wild-type littermates. These results are usually interpreted to suggest that pharmacological modulation of human MDR1 transporters is a feasible strategy to treat multidrug resistant cancer.

However, the high expression in pharmacological barriers in organs that regulate drug distribution, as well as the hypersensitivity of the knockout mice to xenobiotic toxins suggest a major physiological role in the protection of the organism against orally ingested natural toxins. MDR transporters influence drug distribution in three ways: they limit drug absorption in the gastrointestinal tract, they promote drug elimination in the liver, kidney, and intestine, and they regulate drug uptake into cells, tissues, or pharmacological compartments. The gastrointestinal tract, which represents the first line of defense against orally ingested toxins and drugs, is well equipped with a range of ABC transporters that play a prominent role in restricting the passage of hydrophobic compounds that would otherwise cross these barriers by passive diffusion.

Since selective modulation of MDR transporters in cancer cells is difficult to achieve, attempts to circumvent MDR will have to face the profound effects on the distribution of concomitantly administered drugs.

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Mechanism-based Inhibition

Irreversible metabolic inhibition caused by covalent binding of the inhibitor to the enzyme after being metabolized by the same enzyme. The inhibitory effect remains after elimination of the inhibitor from the body.

► Drug Interaction

Meglitinide-related Compounds

Meglitinide contains a benzamide group. Meglitinide-related compounds such as nateglinide are non-sulfonyl-urea oral hypoglycemic drugs used in the treatment of type 2 (non-insulin dependent) diabetes mellitus.

► ATP-dependent K⁺ Channels

► Oral Antidiabetic Drugs

Melanin-concentrating Hormone

Melanin-concentrating hormone (MCH) is a cyclic neuropeptide of 19 amino acids. It is involved in the modulation of feeding behavior. Its actions are mediated by G-protein coupled receptors (MCH1 and MCH2).

► Appetite Control

Melanocortins

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Synonyms

Melanocortins; Melanocyte stimulating hormones

Definition

Melanocortin peptides have been proposed as potent modulators of many pathologies including inflammatory (asthma, arthritis) and cardiovascular disease. They have been shown to be directed against resident cells within tissue such as macrophages, endothelial cells and also circulating leukocytes (neutrophils and lymphocytes). Therefore harnessing their therapeutic potential could lead to the development of novel therapeutics.

Basic Characteristics

History

Melanocortins were identified in common ancestors of lampreys and gnathostomes over 700 million years ago and are little changed throughout evolution. They

are derived from a larger precursor molecule known as the ►**pro-opiomelanocortin (POMC)** protein which contains three main domains: the N-terminus region which contains γ -MSH, the central highly conserved ACTH_{1–39} sequence, with α -MSH at its N-terminus and the C-terminal β -lipotropin, which can be cleaved to generate β -endorphin. Proteolytic cleavage by prohormone converting (PC) enzyme and carboxypeptidases between two pairs of basic amino acids residues (-Lys-Lys-, -Arg-Lys-, -Arg-Arg-, -Lys-Arg-) leads to the generation of biologically active fragments. Identification of their biological potential was first demonstrated by Philip Hench at the Mayo clinic in the 1950's with the parent hormone ACTH_{1–39} being used clinically for the treatment of rheumatoid (RA) and gouty arthritis. However, due to side effects such as adrenal gland suppression, it is no longer in clinical use [1].

Melanocortin Receptors

Melanocortins exert their biological effects by binding to G-protein-coupled seven transmembrane receptors (GPCR's). To date five ►**melanocortin receptors (MCR)** have been identified and termed MC1–5R and shown to display high sequence homology with 60% between MC4R and MC5R and 38% between MC2R and MC4R. All MCR are positively coupled to adenylyl cyclase leading to cAMP accumulation within the target cell. Cloning of them has enabled a greater understanding of their biological roles and identification that a common amino acid sequence His-Phe-Arg-Trp (HFRW) is required for activation [2].

MC1R has many different functions including pigmentation, anti-pyretic and anti-inflammatory actions. It is expressed on an array of cells including melanocytes, macrophages, monocytes, neutrophils, endothelial cells, fibroblasts, mast cells and lymphocytes. α -MSH is the most potent peptide followed by ACTH_{1–39} while β -MSH and γ -MSH cause weak activation. Truncated peptides ACTH_{4–10} and ACTH_{1–10} do not activate MC1R, suggesting that the amino and carboxyl-terminal ends of α -MSH (ACTH_{1–13}) are important for full biological activation of the MC1R.

MC2R is solely activated by ACTH_{1–39} and expressed on the adrenal gland leading to release of steroids by the adrenal cortex. A single study has shown that murine adipocytes show expression something not observed in humans; these findings suggest that MC2R may play a potential role in metabolism.

MC3R is expressed both within the periphery and central nervous system (CNS) with detection in immune cells, gut, placenta but no detection in the adrenal gland or melanocytes. Unlike other MCR, most melanocortins (ACTH_{1–39}, α -, β - and γ -MSH) display equipotent binding and full biological activity with truncated peptides, ACTH_{4–10} and ACTH_{1–10}. MC3R

has been postulated to play a role in modulating food intake and energy metabolism, acute inflammation and ischaemic-damage in the heart. This would suggest that MC3R could be an endogenous control mechanism for modulating these pathologies.

MC4R is solely expressed within the CNS, including the hypothalamus, spinal cord and cortex and binds peptides in a similar fashion to the MC1R. The receptor has been shown to be involved in mediating erectile dysfunction, pain and controlling food intake and energy expenditure and could be an exciting target for controlling obesity.

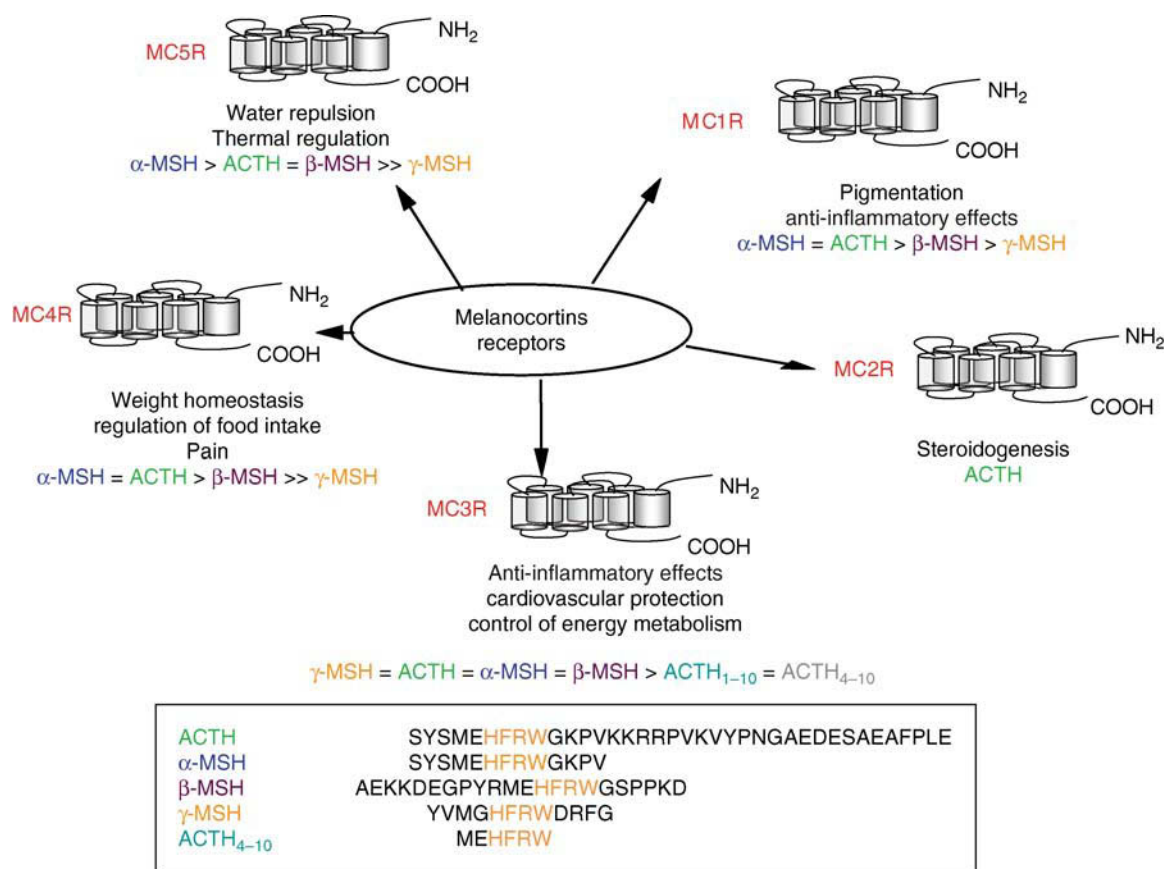
MC5R displays similar binding to MC1R and identified in liver, lung, thymus, testis, ovary, mammary glands, fat cells, bone marrow, skin, skeletal muscle, stomach and duodenum. An interesting observation is its expression in B lymphocytes and in T lymphocytes suggesting a role in immune regulation (Fig. 1).

Drugs

Historically the only melanocortin peptide to be used clinically is the parent hormone from which all these peptides are derived from namely ACTH (see above). It has also been used in the treatment infantile spasms for epilepsy, where it is administered as an intramuscular injection only over a 2–12 weeks period. Obvious side effects include weight gain, puffy face, high blood pressure and an increased risk of infection and should never be administered to patients with diabetics, renal or heart failure. ACTH is also used as a stimulation test to measure adrenal cortex activity, i.e. production of cortisol and is used to ascertain whether someone has ►**Addison's disease**.

Peptides

The development of synthetic and non-peptide mimetics have allowed the unravelling of the biological functions of the MCR expressed in different tissues. This has proved difficult due to the high sequence homology between receptors however we are beginning to see compounds displaying a greater degree of selectivity which will aid scientists in understanding their physiological roles. One of the first synthesised analogues of α -MSH is [Nle⁴,D-Phe⁷]- α -MSH shown to be beneficial in inflammatory models. It is however difficult to imagine that a peptide of this length could have a therapeutic future due to cost to develop and produce, therefore the need to develop smaller compounds was undertaken. The cyclic heptapeptide MTII (chemical sequence: Acetyl-[Nle⁴,Asp⁵,D-Phe⁷, Lys¹⁰]-cyclo- α -Melanocyte Stimulating Hormone Amide Fragment 4–10) a potent MC3/4R agonist has been extensively shown to display anti-inflammatory effects with respect to inhibiting neutrophil migration and also the generation of cytokines/chemokines in models of gouty arthritis and also in controlling metabolic disorders. One of the major breakthroughs in



Melanocortins. Figure 1 Shows the melanocortin receptors, function and naturally occurring peptide sequences that activate them.

melanocortin pharmacology came with the development of SHU9119 which is a substitution of a bulky aromatic amino acid D-Nal(2) into position 7 of ACTH₁₋₃₉ leading to an antagonist at MC3/4R termed SHU9119 (Ac-Nle⁴-c [Asp⁵,D-Nal(2)⁷,Lys¹⁰] α -MSH (4–10)-NH₂). This has allowed many groups to dissect the role of the MC3R in diseases ranging from arthritis to cardiovascular and also in models of obesity. Development of selective MC3R agonists was further enhanced when dTRP⁸- γ -MSH (A highly selective melanocortin type 3 receptor agonist shown to modulate the host inflammatory response in models of experimental gouty arthritis.) was designed and shown to modulate urate crystal induced inflammation in two different rodent models. Other compounds synthesised include PG901 and PG911 developed by substitution of His(6) of the MC3/4R antagonist SHU9119 with Pro(6) or Hyp(6) leading to full agonists at the MC5R and full antagonists at the human MC3/4R these findings highlight that subtle changes of amino acids at discrete positions within the α -MSH sequence can lead to the development of increasingly more selective compounds. We are now entering an era where the Big Pharma has shown an interest in melanocortin biology with the development of non-peptide ligands. These have the

potential to have improved potency, receptor selectivity and bioavailability. The MC4R agonist THIQ (chemical sequence: (N-[(3R)-1,2,3,4-tetrahydroisoquinolinium-3-ylcarbonyl]-(1R)-1-(4-chlorobenzyl)-2-[4-cyclohexyl-4-(1H-1,2,4-triazol-1-ylmethyl)piperidin-1-yl]-2-oxoethylamine) developed by the Merck group displays efficacy in models of erectile dysfunction. In the field of inflammation the MC1R agonist (BMS-470539) developed by Bristol-Myers Squibb has been shown to modulate acute inflammation. BMS-470539 is active at 16.8 and 11.6nM in mice and human melanoma cells, respectively and shown to inhibit LPS induced TNF- α production in BALB/c mice. Palatin Technologies have a novel candidate drug (Bremelanotide formerly known as PT-141) in Phase 2 clinical trials for male and female sexual dysfunction and is believed to be devoid of cardiovascular side effects.

Role of Melanocortin Peptides in Disease Pathologies

Cardiovascular disease affects 2.7 million British people and is a major cause of mortality with circulating neutrophils being proposed as contributing factors in ischaemic damage, though damage can still occur *in vitro* in the absence of blood. Melanocortins have

demonstrated protective effects in myocardial ischaemic-injury (MI) models, where cytokines and neutrophils play a causal role. Short-term ischaemia (5 min) in rats caused by ligation of the left anterior descending coronary artery was reduced by ACTH₁₋₂₄ and the non-selective agonist NDP- α -MSH. Identification of the receptor involved in mediating the protective effects has focused on a panel of pharmacological tools with the selective MC1R agonist ►MS05 being inactive whilst a potential role for MC3R has been proposed, since the MC3/4R antagonist SHU9119 blocked the protective effects of these compounds (ACTH₁₋₂₄ and NDP- α -MSH) within the heart. Recent studies have highlighted that MC3R is the cellular target with the MC3/4R agonist MTII exhibiting protective effects in a more chronic model of IR injury at 24 h post-reperfusion. Murine and rat hearts were shown to express message and protein for MC3R with expression unaltered following IR. These studies propose a previously unrecognised protective role for MC3R activation and may open up new avenues for therapeutic intervention against heart, and possibly other organ IR injury [3].

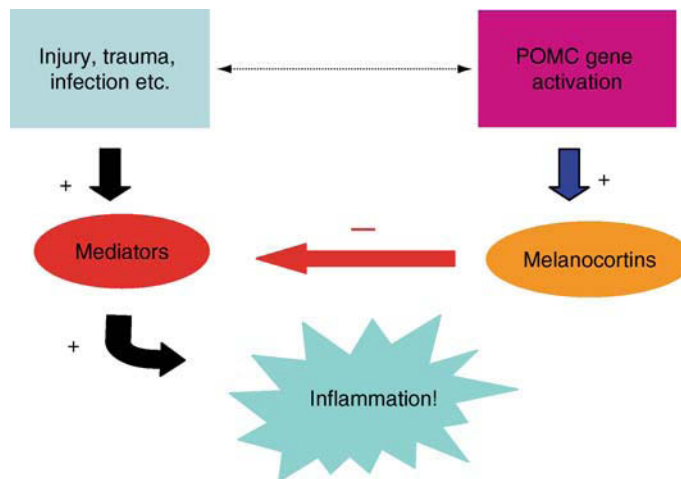
Rheumatoid arthritis (RA) is a complex pathology, which not only affects the joints but also the hearts of patients. There is a 40% mortality rate with RA patients due to cardiovascular complications, with RA patients having elevated cholesterol, blood pressure, diabetes and a tendency to be obese. Therapeutic intervention in RA can lead to some of these complications, with the non-steroidal anti-inflammatory drug Ibuprofen potentially causing a rise in blood pressure. Glucocorticoids can increase cardiovascular problems with acceleration in the development of arterial thickening and narrowing. Disease modifying drugs such as methotrexate have been shown to promote heart disease due to increases in homocysteine. Based on these findings and the fact that the elderly cannot tolerate drugs such as non-steroidals due to decrease in renal clearance, the development of novel naturally occurring therapeutics is essential. A potential alternative to existing therapies could be melanocortin peptides, as they have been demonstrated to possess an array of anti-inflammatory effects in models of arthritis and ischaemic-reperfusion injury.

A clinical study by Catania and colleagues [4] highlighted that α -MSH was elevated in the synovial fluid of patients with RA. An increase in α -MSH concentration was detected in the synovial fluid but not in the plasma suggest local production and potential for an endogenous anti-inflammatory loop within the joint to control the host inflammatory response. Elevation of α -MSH within the knee joint led to a lower degree of inflammation observed in patients. Further work has shown in a pre-clinical model of RA (adjuvant induced arthritis) that α -MSH, was able to modulate weight loss, arthritic score, joint damage and swelling, features

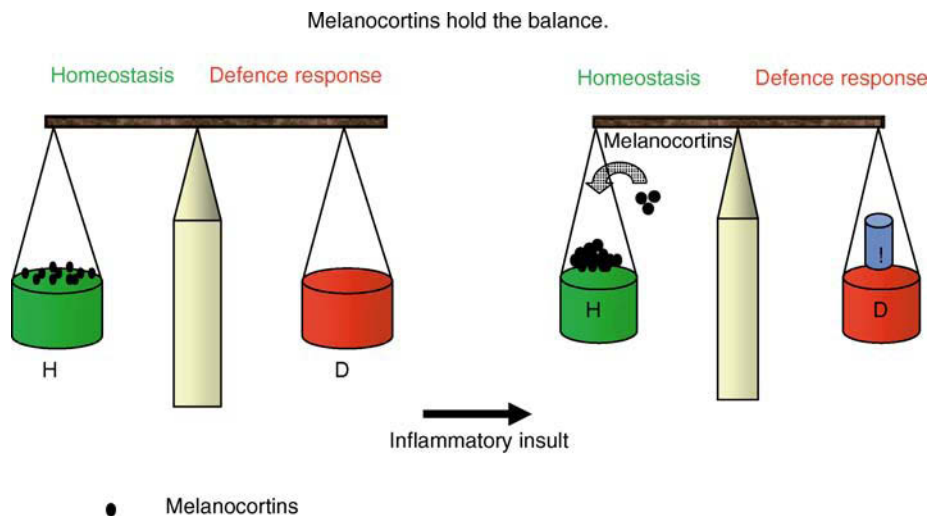
observed in the human pathology. More importantly was the fact that α -MSH caused an increased beneficial effect compared to the clinically used glucocorticoid prednisolone and inhibited glucocorticoid induced weight loss.

Gout: Gouty arthritis is a neutrophil driven pathology which is caused by shedding of urate crystals in the intraarticular and periarticular space of the knee joint resulting in an intense inflammatory response (erythema and oedema). More importantly with an increasing ageing and obese population – both risk factors in gout, we are potentially facing a financial and debilitating time-bomb. Melanocortin peptides have been shown to modulate neutrophil migration and release of pro-inflammatory cytokines and chemokines in a ►corticosterone independent manner. This inhibitory effect was due to blockade of inflammatory mediator release from macrophages via activation of MC3R. Further studies using the naturally occurring γ_2 -MSH, partially-selective synthetic peptide MTII and highly selective MC3R agonist ►dTRP⁸- γ -MSH have also highlighted the importance of MC3R in gouty arthritis. Recently MC3R null mice have been evaluated in models of gout and have shown an exacerbation in the host inflammatory response to urate crystals compared to wild types. This data further strengthens the hypothesis that MC3R is preferential in modulating the host inflammatory response compared to MC1R (Fig. 2).

Obesity and Energy Expenditure: Obesity is fast becoming a major epidemic within our society. A number of reasons have been attributed to this including a lack of exercise, an excess of readily available food and genetic factors; all these result in an inability to maintain a stable body weight. To restore homeostasis, a number of alterations to our lifestyle need to be addressed: more exercise, reduction in fast, readily available food or therapeutic intervention. The arcuate nucleus of the hypothalamus is a region where neurons contain the *POMC* gene. During periods of starvation or overeating, activation of POMC neurons is reduced or activated respectively, thus maintaining homeostatic balance and helping us control body weight. To mediate this effect, α -MSH derived from POMC stimulates the central MCR whilst antagonism occurs via AgRP. Fasting stimulates AgRP neurons whilst starvation causes inhibition; these effects occur *via* central MCR (MC3 and 4R) and maintain energy homeostasis. Utilisation of knock-out animals has allowed for the role of these receptors to be dissected. MC3R null mice have increased adipose tissue and are hypoactive, whilst POMC null mice are obese. The pharmaceutical industry has mainly focused on the MC4R as a major target for eating disorders. Chronic antagonism of MC3/4R using SHU9119 and/or AgRP results in obesity by increasing food intake and decreasing energy expenditure whilst MTII and α -MSH has the opposite effect. Therefore at present it would appear that MC3R is involved in regulating adiposity and



Melanocortins. Figure 2 Shows anti-inflammatory effect of melanocortin peptides.



Melanocortins. Figure 3 Melanocortins have the potential to restore homeostasis to tissue.

MC4R for feeding behaviour, targeting one or both of these receptors in addition to understanding the complex cross-talk within the CNS may lead to the development of melanocortin based therapeutics for the treatment of eating disorders [5].

Conclusion

Melanocortin peptides are potent anti-inflammatory agents displaying beneficial effects in diseases ranging from cardiovascular to arthritis to obesity to name a few. Within an inflammatory context, they have the ability to switch off early production of cytokines and at later stages they increase levels of anti-inflammatory proteins that lead to the resolution of the host inflammatory response potentially restoring homeostasis to the tissue. They could eventually be viewed as an alternative to glucocorticoids, as their mode of action often resembles that seen

with this widely used therapeutic but may be devoid of the side effects associated with continuous glucocorticoid therapy. The difficulty with peptide therapeutics is their short half-lives due to rapid clearance, in some respects this maybe of benefit as they will not accumulate within the body, thus reducing potential side effects. Development of non-peptide MCR agonists may hold the key and lead to the development of the first melanocortin based therapeutics (Fig. 3).

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Melanocyte-stimulating Hormone

Melanocyte-stimulating hormones (α MSH, β MSH, γ MSH; also called melanocortins or melanotropins) are generated by proteolytic cleavage of proopiomelanocortin. MSH isoforms increase the synthesis of eumelanin by melanocytes and regulate body weight by activating anorexigenic pathways in the hypothalamus.

- Appetite Control
- Melanocortins

Melatonin

Melatonin is a hormone secreted by the pineal gland. Production of melatonin is regulated via signals from the suprachiasmatic nucleus, the seat of a mammal's circadian clock. Thus, melatonin levels are highest during the hours of darkness, and lowest during the day (i.e. light). Exogenously administered melatonin elicits a sleep promoting effect, although owing to interaction with the fluctuations of endogenous melatonin, this effect is highly dependent on the time of day the compound is ingested. Nevertheless, a synthetic compound that acts as an agonist at melatonin receptors (ramelteon) has been shown clinically to be useful as a *hypnotic*. Endogenously, in addition to its role in the circadian cycle, melatonin acts as an antioxidant and immunoregulator.

- Sleep

Membrane Signal Transduction

- Transmembrane Signaling

Membrane Transport Protein

- Table appendix: Membrane Transport Proteins

Menaquinones-n

- Vitamin K

Ménière's Disease

Ménière's disease is a condition in which there is increased volume of endolymph with dilatation of the membranous labyrinth. It is brought about by excessive production of endolymph or impaired outflow from the labyrinth. It is characterised by attacks of vertigo, tinnitus, nausea and vomiting.

- Emesis

Mesangial Cells

Mesangial cells are the smooth muscle-like cells of the capillaries in the glomerulus of the kidney.

Mesolimbic System/Reward System

In 1954, experiments by Olds and Milner revealed that the brain has specialized “centers” for reward functions. In these studies electrical stimulation of certain brain sites was found to be highly rewarding in the sense that rats operantly respond for electrical stimulation of these brain sites, often to the exclusion of any other activity. A neurotransmitter system that is particularly sensitive to electrical self-stimulation is the mesolimbic dopamine projection that originates in the ventral tegmental area and projects to structures closely

associated with the limbic system, most prominently the nucleus accumbens shell region and the prefrontal cortex. Because of its ubiquitous involvement in the regulation of reward-related behavior this system has been characterized as a neurochemical substrate of reward.

- Drug Addiction/Dependence
- Dopamine System

Metabolic Syndrome

The metabolic syndrome (also called syndrome X) is characterized by several clinical features which are associated with an increased risk for cardiovascular diseases. Two classification systems by the National Cholesterol Education Program (NCEP) as well as by the World Health Organization (WHO) have been used to define the metabolic syndrome (see table). The most important features are abdominal obesity, insulin resistance with impaired glucose regulation, dyslipidemia (high triglyceride and low HDL cholesterol plasma levels), and hypertension. The prevalence of the metabolic syndrome is increasing dramatically worldwide. It is generally believed that the increase in adipose tissue is the primary and most important mechanism underlying the development of the metabolic syndrome. There is good evidence that an increase in the visceral fat is linked to insulin resistance. Potential mechanisms linking obesity to insulin resistance and the development of type 2 diabetes are an increase in the circulating levels of free fatty acids which decrease insulin sensitivity as well as the altered production of

adipokines such as adiponectin or resistin. While adiponectin has beneficial effects on insulin sensitivity of peripheral organs, resistin can induce insulin resistance. In patients with metabolic syndrome, adiponectin plasma levels are reduced whereas resistin levels are increased. The increased availability of free fatty acids derived from adipose tissue results in an accelerated synthesis of very low-density lipoproteins containing triglycerides in the liver of patients with metabolic syndrome. High triglyceride concentrations in ApoB-containing lipoproteins are typically accompanied by a decrease in HDL cholesterol levels due to a decreased exchange of triglycerides and cholesterol esters between ApoB-containing lipoproteins and HDL particles via the cholesterol ester transfer protein (CETP). There is also evidence that hyperinsulinemia can result in arterial hypertension due to increased sodium retention and an increase in the activity of the sympathetic nervous system as well as by direct vascular effects. Recently, evidence has emerged that obesity is accompanied by a chronic inflammatory process in the fat tissue resulting in increased expression of TNF α and other cytokines which may contribute to the systemic insulin resistance and which may further promote the chronic inflammatory processes underlying the development of atherosclerosis. Thus, the metabolic syndrome comprises a variety of symptoms and processes which are interconnected in a complex manner and eventually promote the development of atherosclerotic processes resulting in an increased cardiovascular morbidity.

- Diabetes Mellitus
- Adipokines
- Orexins
- Lipoprotein Metabolism
- Antihypertensive Drugs
- Antiobesity Drugs

Criteria for diagnosis of the metabolic syndrome

Clinical features	NCEP ATPIII criteria ≥ 3 of the criteria below	WHO criteria impaired glucose regulation/insulin resistance and ≥ 2 other criteria
Impaired glucose regulation/insulin resistance	Fasting plasma glucose ≥ 110 mg/dl	Type 2 diabetes mellitus <i>or</i> impaired fasting glycemia [≥ 6.1 mmol/l (110 mg/dl)] <i>or</i> impaired glucose tolerance <i>or</i> glucose uptake below lowest quartile under hyperinsulinemic, euglycemic conditions
Abdominal obesity	Waist circumference >102 cm (40 in.) in men, >88 cm (35 in.) in women	Waist/hip ratio >0.90 in men, >0.85 in women <i>or</i> body mass index >30 kg/m ²
Hypertriglyceridemia	≥ 150 mg/dl	≥ 1.7 mmol/l (150 mg/dl)
Low levels of HDL cholesterol	<40 mg/dl in men, <50 mg/dl in women	<0.9 mmol/l (35 mg/dl) in men, 1.0 mmol/l (39 mg/dl) in women
High blood pressure	$\geq 130/85$ mmHg	$\geq 140/90$ mmHg
Microalbuminuria	Not included	≥ 20 μ g/min <i>or</i> albumin: creatinine ratio ≥ 30 mg/g

Metabotropic Glutamate Receptors

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Synonyms

G-protein coupled glutamate receptors

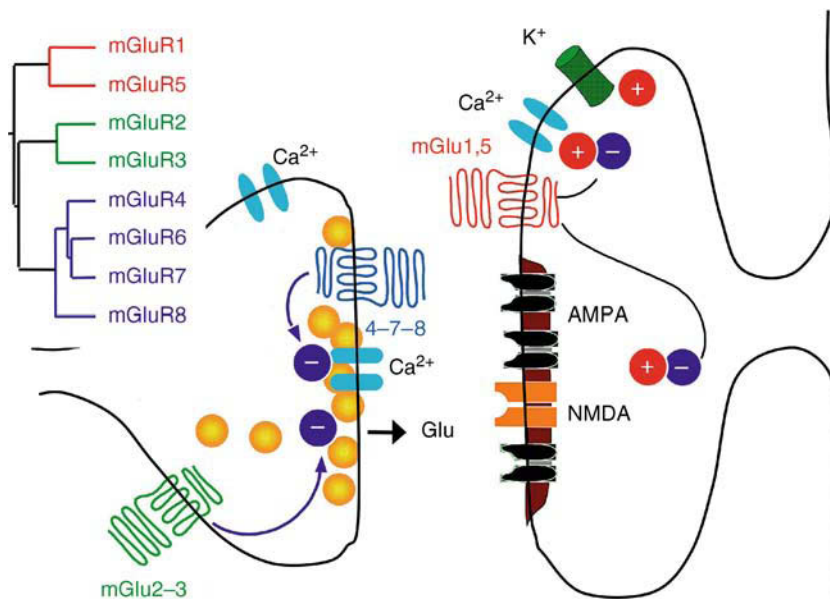
Definition

► **Glutamate** is the transmitter of most fast excitatory synapses in the central nervous system. To excite (depolarize) the post-synaptic neuron, glutamate activates various types of receptor channels (the ► **ionotropic glutamate (iGlu) receptors**) known as the NMDA, AMPA and kainate receptors (Fig. 1). Glutamate also acts on receptors called the ► **metabotropic glutamate (mGlu) receptors** that are coupled to heterotrimeric G proteins to activate intracellular pathways. Eight mGlu receptors have been identified in mammals and all play important roles in the fine-tuning of most glutamatergic as well as other synapses [1].

Basic Characteristics

The mGlu Receptor Family

The mGlu receptors were first identified as receptors responsible for the glutamate stimulation of phospholipase C (PLC) in neurons. Latter, other mGlu receptors were shown to inhibit ► **adenylyl cyclase (AC)**. Molecular cloning and the sequencing of mammalian genomes identified eight genes encoding mGlu receptors [1]. Additional mGlu receptor subtypes exist due to alternative splicing of the mRNA encoding mGlu1 (splice variants a, b and d are the best characterized), mGlu5 (a and b), mGlu7 (a and b) and mGlu8 (a and b). All these splice variants differ in the sequence of their carboxy-terminal intracellular tail. These receptors can be classified into three groups based on their sequence similarity, their pharmacology (see below) and their transduction mechanism [1]. The sequence similarity is ~60–70% between members of a given group, and drops to 40% between receptors of two distinct groups. The group-I is composed of mGlu1 and mGlu5 receptors, which are both coupled to the stimulation of PLC. The mGlu2 and mGlu3 receptors form the group-II, whereas the mGlu4, mGlu6, mGlu7 and mGlu8 constitute the group-III. Both group-II and group-III mGlu receptors can inhibit AC in transfected cells.



Metabotropic Glutamate Receptors. Figure 1 The mGlu receptors and the fine tuning of glutamatergic synapses. Release of glutamate from nerve terminals activates the iGlu receptors (the NMDA and AMPA subtypes) in the post-synaptic element, leading to neuronal excitation. The group-I mGlu receptors (mGlu1 or mGlu5) located on the side of the post-synaptic element regulates the activity of Ca^{2+} and K^{+} channels as well as of iGlu receptors via intracellular pathways. The group-II mGlu2 and mGlu3 receptors regulate not only the release of glutamate when located on the pre-synaptic terminal, but can also participate in the glutamate response when located in the post-synaptic element. Most group-III mGlu receptors (4, 7 and 8) are located in the pre-synaptic release zone where they inhibit glutamate release. Inserted in the left top corner is a dendrogram of the mGluR family, illustrating the three groups of mGluRs.

Transduction Mechanism and Function of mGlu Receptors

Group-I mGlu receptors are mainly found on the side of the post-synaptic element (Fig. 1). By activating PLC, they stimulate the formation of inositol triphosphate leading to the release of Ca^{2+} from intracellular stores. This Ca^{2+} signal can then regulate the activity of enzymes such as protein kinase C and phospholipase A2. Group-I mGlu receptors can also regulate the activity of various channels, including Ca^{2+} channels. They also inhibit or activate K^+ channels leading either to a decrease or to an increase in neuronal excitability. Group-I mGlu receptors also modulate (mainly positively) the activity of the iGlu receptors of the AMPA and NMDA types, and can therefore modulate the excitatory effect of glutamate. NMDA receptors are known to be responsible for the ►excitotoxic effect of ►glutamate and to play an important role in synaptic plasticity including ►long-term potentiation (LTP) and long-term depression (LTD). It is therefore not surprising that antagonists of group-I mGlu receptors have neuroprotective effects and can inhibit synaptic plasticity such as LTP and LTD. By stimulating the release of endocannabinoids from the post-synaptic element, activation of group-I mGluRs can also have indirect pre-synaptic effect, inhibiting glutamate release.

The mGlu1 receptors are expressed at high density in the Purkinje neurons in the cerebellum where they are involved in a synaptic plasticity phenomenon important for the control of eye movements. These receptors are also found in the thalamus, the olfactory bulb and in some neurons of the hippocampus. The mGlu5 receptors are highly expressed in the pyramidal neurons of the hippocampus where they regulate a LTP phenomenon important for spatial memory. The mGlu5 receptors are also found in glial cells, but their function in these cells is still not yet fully elucidated. Both group-I mGlu receptors are expressed in many other brain regions and as such are involved in many processes such as the central response to pain, the control of movements via the extrapyramidal motor circuit. Group-I mGlu receptors are also found outside the central nervous system, such as in the sensory terminals in the skin where they can be at the origin of the inflammatory pain sensation. These various observations help in understanding the anxiolytic, antiparkinsonian and analgesic effects, as well as the decrease in the development of cocaine dependence observed with a mGlu5 receptor antagonist (MPEP (2-Methyl-6-(Phenylethynyl) Pyridine)) [2]. In addition neuroprotective and antiepileptic effects have been observed with an mGlu1 receptor antagonist (BAY367620).

Group-II mGlu receptors have been observed in pre-synaptic as well as in post-synaptic elements. These receptors can inhibit AC activity, but their main action

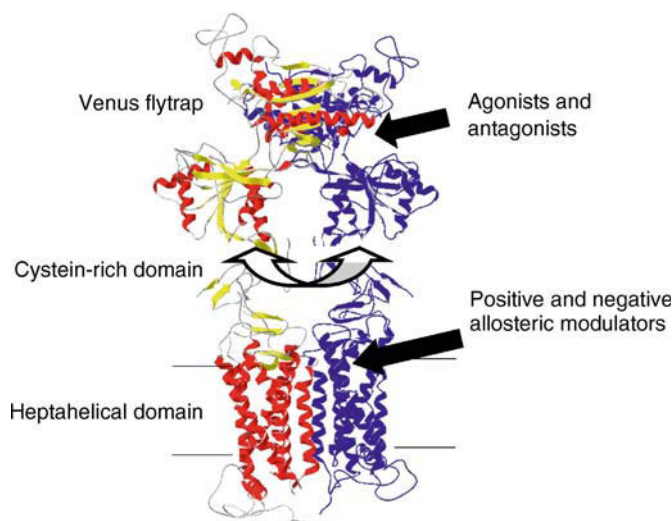
in neurons is to regulate the activity of various types of channels, including inhibition of Ca^{2+} channels (and as such they can inhibit the release process at the pre-synaptic level). In the brain, mGlu2 receptors are highly expressed in Golgi cells in the cerebellum and in the dentate granule neurons of the hippocampal formation where they are involved in a LTD phenomenon. The other group-II mGlu receptor, mGlu3, is found not only in neurons, but also in astrocytes where its role is not yet elucidated. The mGlu3 receptor is mainly expressed in the reticular nucleus of the thalamus. Group-II mGlu receptors are also expressed in many other brain areas, and the recent development of selective and systematically active group-II mGlu receptor agonists (such as LY354740) reveal the potential use of such compounds for the treatment of anxiety, schizophrenia and drug dependence [3].

Group-III mGlu receptors are mostly found in the pre-synaptic element within the synaptic release site (Fig. 1), except the mGlu6 receptor, which is responsible for the post-synaptic action of glutamate on the ON bipolar cells in the retina. These receptors are therefore considered as autoreceptors controlling the glutamate release process. The mGlu7 and 4 receptors are also found in GABA-ergic terminals where they inhibit the release of the inhibitory transmitter GABA. Like group-II receptors, group-III mGlu receptors can inhibit AC activity, but their main action is the inhibition of Ca^{2+} channels leading to the inhibition of the release process. Due to their localization at the synaptic vesicle release site, it is likely that group-III mGlu receptors can directly control the release machinery.

The mGlu7 receptor type is widely expressed in the brain and is activated by high concentration of glutamate (100–1,000 μM). It may serve to prevent over-activity of glutamatergic synapses, and accordingly, the deletion of its gene in mice leads to the rapid development of general epilepsy and death. The mGlu4 receptor is mostly expressed in the cerebellum and the olfactory bulb, but also in other areas. It has been shown to play a role in certain types of absence epilepsy. The mGlu8 receptor is expressed in some restricted areas in the brain and its physiological function is not yet elucidated, but the deletion of the mGlu8 gene result in increased anxiety and weight gain.

Original Structure and Activation Mechanism of mGlu Receptors

Alike any other G-protein coupled receptors (GPCRs), mGlu receptors have seven transmembrane helices, also known as the ►heptahelical domain (Fig. 2). As observed for all GPCRs, the intracellular loops 2 and 3 as well as the C-terminal tail are the key determinants for the interaction with and activation of G-proteins. However, sequence similarity analysis as well as specific structural features make these mGlu receptors different from many other



Metabotropic Glutamate Receptors. Figure 2 The original structure and activation mechanism of mGlu receptors. These receptors dimeric entities, each protomer (one in red and yellow, the other in blue) being composed of a Venus Flytrap (VFT) domain connected to a heptahelical domain via a cysteine-rich region. Binding of agonists in the VFT cleft stabilizes a closed state of the VFT. This is associated with a relative movement of the two VFTs in the dimer, as indicated with the double white arrow. Antagonists act by preventing closure of the VFT. Allosteric modulators interact in the heptahelical domain. These can either act as non-competitive antagonists (also called negative allosteric modulators) or facilitate agonist activation of the receptor (positive allosteric modulators).

GPCRs, and were the first members of the class C GPCRs. The main structural originality of these receptors is their large extracellular domain (Fig. 2) that contains the glutamate binding site [4]. The structure of this extracellular domain has been solved for both mGluR1, mGluR3 and mGluR7 [4]. It is composed of two lobes that are separated by a wide cleft in the absence of glutamate. Upon binding of the agonist within this cleft, the two lobes close like a Venus Flytrap trapping an insect. This domain is therefore often called the “Venus Flytrap” (VFT) domain (VFT domain), and is connected to the first transmembrane segment of the ▶heptahelical domain by a cysteine-rich domain that plays a critical role in transmitting the signal from the VFT (VFT domain) to the ▶heptahelical domain (Fig. 2). The second original feature of the mGlu receptors compared to other GPCRs is that they are always found as dimers in the brain. A disulfide bond linking the two VFTs stabilizes this dimeric structure. Today, only homodimers have been identified. This dimeric structure of mGlu receptors is probably critical for glutamate to activate the receptor. Indeed, the resolution of the structure of the extracellular domain of mGlu1 and mGlu3 revealed a dimer of VFTs, and a large change in the general conformation of the dimer is seen in the presence of glutamate. This change is such that the C-terminal ends of the two VFT modules become closer in the presence of agonists (Fig. 2). Because the C-terminal ends on the VFT modules are linked to the

heptahelical domain via the cysteine-rich region, such a change of conformation is likely to induce a change of the relative position of the two heptahelical domains leading to the activation of only one of them which in turn activate the G-protein.

As we will see below, this original and complex structure of the mGlu receptors offers multiple possibilities to develop drugs modulating their activity.

Drugs

All compounds identified so far acting at the glutamate binding site share a glutamate-like structure. Such compounds are either agonists or competitive antagonists (Fig. 2). Within the last 15 years a number of group-selective compounds have been characterized. However, due to the high conservation of the glutamate binding site between receptors of the same group, very few subtype selective compounds have been characterized [3]. By using systematic screening procedures, pharmaceutical industries have identified ▶allosteric modulators of several mGlu receptor subtypes [2, 5]. Such molecules have a structure very different from glutamate and do not bind to the VFT but rather in the heptahelical domain (Fig. 2). These can be either non-competitive antagonists [2] or positive ▶allosteric modulators [5] and are subtype selective. Such compounds offer new possibilities to modulate mGlu receptor activity in the brain. Most importantly, because compounds activating mGlu receptors are expected

to have multiple therapeutic applications (see above), positive allosteric modulators may be more efficacious than pure agonists since they are less likely to induce receptor desensitization. Moreover, in contrast to pure agonists that activate the receptor all the time and in every regions where the receptor is expressed, positive allosteric modulators only facilitate receptor activation by endogenous ligands. As such positive allosteric modulators maintain the normal biological activity of the receptor and its activation only where needed. As such, positive allosteric modulators are expected to have far less side effects, facilitating their use in a wider range of concentrations.

Group-I mGlu Receptor Compounds

Quisqualate was the first compound shown to activate group-I mGlu receptors. Although it is also active on the AMPA type of iGlu receptors, it remains the most potent group-I agonist. 1S,3R-ACPD was the first selective mGlu receptor agonist identified, but it activates group-I, group-II and some group-III mGlu receptors. ACPD (1-aminocyclopentane-1,3-dicarboxylic acid) is identified as the mGlu selective agonist. Within the four stereoisomers, 1S,3R-ACPD activates group-I and group-II mGlu receptors as well as some group-III receptors (mGlu8) at higher concentrations. The 1S,3S-ACPD isomer is one of the first selective group-II mGlu receptor agonists described. These molecules have been widely used to identify the possible physiological functions of mGlu receptors.

3,5-DHPG remains the best characterized selective group-I agonist, though it has a low potency (10 μ M). A few competitive group-I antagonists have been identified (the first was MCPG, which is also active on group-II receptors) such as LY367366 and LY393675, which are acting on both mGlu1 and mGlu5, and LY367385 which is mGlu1 selective, but all have a very low affinity. As mentioned above, non-competitive antagonists have been identified for group-I mGlu receptors that bind within the heptahelical domain. These are MPEP (2-Methyl-6-(Phenylethynyl) Pyridin), selective for mGlu5, and BAY367620, selective for mGlu1. These highly selective and potent compounds have helped in identifying the important physiological roles of group-I mGlu receptors mentioned above.

Other molecules also acting within the heptahelical domain of mGlu1 and mGlu5 have been shown to potentiate the action of glutamate and are therefore positive allosteric modulators. Such compounds have no effect on their own but increase both the efficacy and potency of agonists. These are Ro01-6128 and CPPHA for mGlu1 and mGlu5, respectively [5]. No behavioural effect has already been reported for these molecules but according to the known function of group-I mGlu receptors, positive action on cognition are expected.

Group-II mGlu Receptor Compounds

Potent and selective compounds have been identified for group-II receptors. The first one was DCGIV, but this compound also antagonizes group-III mGlu receptors and activates NMDA receptors limiting its usefulness in in vivo studies. LY354740 and its analogue LY379268 are actually the best group-II agonists, and, most interestingly, they are systemically active and have unravelled the various possible applications of group-II agonists described above. As for group-I mGluRs, positive allosteric modulators have been identified for mGlu2. These compounds also interact in the heptahelical domain and are very selective for mGlu2 (X). As observed with the group-II agonists, these modulators also have antipsychotic and anxiolytic activities, demonstrating that the behavioural effects of group-II agonists mostly result from the activation of mGlu2 receptors.

MCPG is the first described antagonist acting at group-II mGlu receptors but it is not selective, being also an antagonist of group-I and some group-III receptors. Today, the commonly used group-II competitive antagonists are derivatives of the non-selective mGlu agonist L-CCG-I: LY341495 > XE-CCG-I > PCCG4 > MCCG-I. However, LY341495 is active at all mGlu receptors, being more potent not only at group-II but also at most group-III receptors (mGlu6, 7 and 8). Moreover, the activity of the other compounds on group-III receptors remains to be characterized. Today, non-competitive antagonists acting in the heptahelical domain of group-II mGluRs have not yet been reported.

Group-III mGlu Receptor Compounds

L-AP4 and the natural compound, L-SOP, were the first identified group-III agonists. More recently, Z-CPrAP4, ACPT-I, (+)-PPG and DCPG were shown to be additional group-III agonists. DCPG, being 50–100 times more potent on mGlu8 than on the other group-III receptors, is proposed as a selective mGlu8 agonist. Among these molecules, ACPT-I was recently shown to pass the blood brain barrier, allowing easier in vivo studies, and demonstrating antiparkinsonian and antidepressant-like effect of this molecule. Modulators acting in the heptahelical domain of group-III mGluRs have also been identified. PHCCC was found to be a positive allosteric modulator selective for mGlu4 and display antiparkinsonian activity, and AMN082 was reported as an mGlu7 selective ligand. Although the latter also binds in the heptahelical domain of mGlu7, it directly activates the receptor even in the absence of glutamate.

Today, the number of group-III antagonists is rather limited. The only selective compounds are MAP4, MSOP and CPPG.

Currently, more group-selective and subtype selective compounds remain to be discovered. The recent demonstration of a possible action of new subtype selective

group-I modulators opens a new route for the discovery of such molecules for either group-II or group-III receptors. These will certainly be useful for the identification of the physiological roles of these different receptors and for the discovery of new therapeutic drugs.

► Iontropic Glutamate Receptors

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Metabotropic Receptor

For differentiation of G-protein-coupled receptor subtypes from subtypes permanently linked to ion channels (ligand-gated ion channels) the terms metabotropic versus ionotropic receptors, respectively, are used. Prime examples of metabotropic receptors are given by the mGlu_{1–8} receptor family of G-protein-coupled glutamate receptors.

► Transmembrane Signaling

Metalloprote(in)ases

Metalloproteinases are a subgroup of proteinases. They are responsible for the cleavage of peptide bonds within a protein (proteolysis). Metalloproteinases contain a metal ion in the active center and are divided into four subclasses dependent on their mechanism of catalysis.

- Non-viral Peptidases
- Matrix Metalloproteases

Methicillin-resistant Staphylococci

Methicillin-resistant staphylococci are strains of staphylococci, which show resistance to a wide variety of antibiotics. They are named for their resistance to methicillin, a β -lactamase-resistant penicillin. Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a serious problem particularly in hospitals.

N-Methyl D-aspartate Receptors

► Iontropic Glutamate Receptors

Methylating Agents

► Alkylating Agents

3,4-Methylenedioxymethamphetamine

3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) is a synthetic analog of amphetamine that produces hallucinations, an elevation in mood, and a feeling of “emotional closeness”. This latter property has led to Ecstasy being referred to as the “hug drug”. The unique properties of Ecstasy as compared to the parent compound amphetamine are believed to be due to the more selective effects of Ecstasy in promoting transporter-mediated release of serotonin. The use of Ecstasy has become a part of the culture associated with “rave” style dance parties.

► Psychostimulants

2-Methyl-6-(phenylethynyl) Pyridine

MPEP (2-methyl-6-(phenylethynyl) pyridine) is the best characterized mGlu5 selective non-competitive antagonist. This compound was one of the first of the

allosteric regulators of the mGlu receptor identified as a non-competitive antagonist. MPEP is systematically active and has been very helpful in identifying the role of the mGlu5 receptor subtype, including its involvement in inflammatory pain, anxiety and the development of cocaine addiction.

► Metabotropic Glutamate Receptors

1-Methyl-4-Phenylpyridium

1-methyl-4-phenylpyridinium (MPP^+), a permeant organic cation, is an excellent substrate for all organic cation transporters, but also for vesicular (VMAT) and some neuronal (e.g. DAT, NET) monoamine transporters. It is a potent neurotoxin and produces Parkinson's disease-like symptoms. It is generated in vivo from MPTP (1-methyl-4-phenyl-tetrahydropyridinium).

► Organic Cation Transporters

1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a toxic agent which selectively destroys nigrostriatal neurons, but does not effect dopaminergic neurons elsewhere. MPTP is converted to its toxic metabolite, MPP^+ , by the enzyme monoamine oxidase. MPP^+ is taken up via dopamine transporters and acts selectively on dopaminergic neurons by inhibiting mitochondrial oxidation reactions, which eventually results in the destruction of the cell. MPTP produces a severe form of Parkinson disease in primates.

► Nigrostriatal Tract/Pathway

Methylxanthines

Methylxanthines are naturally occurring drugs, including theophylline, theobromine and caffeine. Methylxanthines at relatively high doses inhibit phosphodiesterases, which results in an increase in intracellular cAMP

levels. Inhibition of phosphodiesterase may be responsible for the tachycardia and relaxation of smooth muscles observed in response to methylxanthines. Already at lower doses, methylxanthines act as antagonists on adenosine receptors. The latter effect is believed to be responsible for the psychomotor stimulant effects of methylxanthines. Theophylline is used clinically as an anti-asthmatic drug, due to its strong bronchodilator effects.

► Phosphodiesterase

► Adenosine Receptors

MHC

► Major Histocompatibility Complex

MIC

► Minimal Inhibitory Concentration

Michaelis–Menten Kinetics

In 1913 L. Michaelis and M.L. Menten realized that the kinetics of enzyme reactions differed significantly from the kinetics of conventional chemical reactions. They put the reaction of substrate plus enzyme yielding enzyme plus substrate into the form of the equation: reaction velocity = (maximal velocity of the reaction $\times$ substrate concentration) / (concentration of substrate + a fitting constant K_m). This latter constant characterized the tightness of the binding of the reaction between substrate and enzyme or the concentration at which the reaction was half the maximal value. This equation is formally identical to the Langmuir adsorption isotherm that relates the binding of a chemical to a surface. Both of these models form the basis of drug–receptor interaction, thus the kinetics involved are referred to as “Michaelian,” or “Langmuirian” in form.

► Drug–Receptor Interaction

Microarray

A microscopic, ordered array of nucleic acids, proteins, small molecules, cells or other substances that enables parallel analysis of complex biochemical samples.

- Gene Expression Analysis
- Microarray Technology

Microarray Hybridization

Microarray hybridization is a process by which nucleic acids are detected by hybridizing with complementary sequences bound to wafers at specific array coordinates. Hundreds to thousands of gene products may be measured in a single experiment.

- Microarray Technology
- Gene Expression Analysis

Microarray Technology

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Synonyms

Biochip; Gene array; Gene chip

Definition

A method to study the activity of a large numbers of genes simultaneously. This method uses high speed robotics to precisely apply tiny droplets containing biologically active material (DNA, proteins, chemicals) to glass slides. In Functional Genomics DNA microarrays are used to monitor gene expression at RNA level, to identify genetic mutations and to discover novel regulatory regions in genomic sequences. Protein or chemical arrays are used to monitor protein expression and to identify protein–protein or drug–protein interactions. Typically tens of thousands of genes or interactions can be monitored in a single experiment.

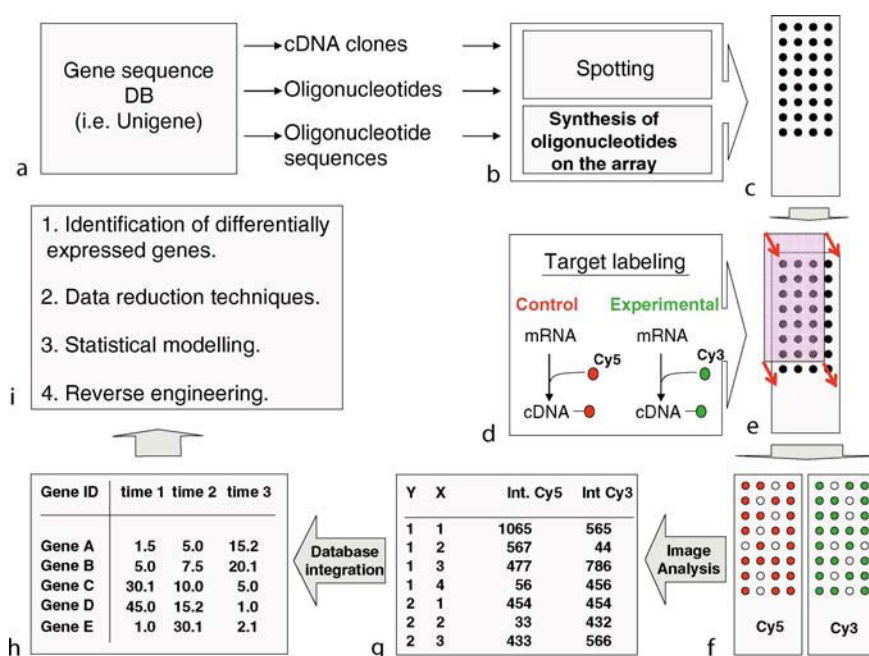
Description Gene Expression Profiling

The most common application of microarray technology is in monitoring gene expression (gene expression profiling).

The principle: The technique is based on a classic molecular biology procedure called reverse Northern blot. mRNA is extracted from a biological sample and reverse transcribed in the presence of a radioactive or fluorescent precursor. The reaction produces a pool of labeled complementary DNA copies (cDNAs) representative of the original mRNA pool and it is defined here as ►target. The expression of an individual gene is quantified by hybridizing the target to the gene specific cDNA (defined as ►probe) which has been previously spotted on a solid surface. The amount of radioactive or fluorescent signal associated to the spot is proportional to the amount of gene specific RNA originally present in the cell. Multiple cDNAs can be spotted in an ordered pattern (►array) allowing the quantification of multiple genes. Before the advent of microarray technology researchers were employing low complexity nylon-based arrays, manufactured using manual devices. The technology subsequently evolved with the introduction of robotics which made possible to generate reproducible high density nylon filters (50 spots per square centimeter). This technology employed large membranes with up to 50,000 DNA spots, required several milliliters of hybridization solution and furthermore was relatively time consuming. The introduction of high-precision and high-speed robotics brought to a further miniaturization of the technology with the production of microarrays (up to 10,000 DNA spots per square centimeter).

Because of the high density of signals in the processed arrays the conversion of the radioactive or fluorescent emissions in a numeric value is performed by sophisticated image analysis software which identifies the position of every spot in the array, determines the spot and the local background intensities, and derives a number representing a spot-specific signal (Fig. 1h). Spot coordinates are then associated to a gene identities within the image analysis program or in a specific gene expression profiling database (Fig. 1i). Differentially expressed genes are identified comparing two or more arrays derived from different biological samples. When a gene is represented by multiple spots in an array or replicated hybridizations are available, statistical tests can be used to assess the significance of the observed differences. Furthermore, data mining techniques are used to identify groups of genes with similar expression profiles across different samples (Fig. 1j).

Array types: There are two main types of arrays: (i) oligonucleotide and (ii) cDNA arrays. In the first type short 20–25mers are synthesized on a silica chip



Microarray Technology. Figure 1 Microarray technology applied to gene expression profiling. The figure summarizes the process from the construction of the microarray to data analysis. (a) This panel describes the first step in manufacturing a microarray for gene expression profiling: the choice of the cDNA clones or the oligonucleotide sequences to represent the genes of interest. Clones or sequences are selected from sequence databases such as Unigene. (b) cDNA clones are PCR amplified and spotted on a slide, whereas oligonucleotides can be synthesized and spotted or synthesized in situ. (c) The result is an array of DNA samples representing several thousands of genes. Thousands of arrays can be manufactured from micrograms of DNA. (d) This panel represents the basic protocol to synthesize a labeled target. In this example, mRNAs derived from experimental and a control samples are differentially labeled, mixed, and (e) hybridized on an array. Typically the hybridization reaction is performed under a coverslip (marked in pink). (f) After hybridization the array is washed and scanned. Two images are produced for each array. (g) An image analysis program identifies the spots and derives a numerical value representing the intensity of the signal subtracted by the local background. The panel represents the output of a typical image analysis software. X and Y are spot coordinates. Int. Cy5 and Int. Cy3 are the signal intensities for each spot in the two channels. (h) Database integration associates a gene identity to each spot and integrates data from different arrays within the experiment. The example refers to a time course. Gene expression intensities for genes A–E are reported for time 1, time 2, and time 3 of a hypothetical experiment using three arrays. Each value is expressed as a ratio between the experimental and the control sample (i) Result of bioinformatics analysis applied to the data is summarized in panel i.

using photolithography (Affymetrix arrays, ►www.affymetrix.com), or using an ink-jet based technology (Rosetta Inpharmatics, ►www.rii.com). Alternatively presynthesized oligonucleotides can be printed onto glass slides. In the second type nucleic acids (usually in a form of PCR products) are robotically printed on glass slides as spots in defined locations. The first glass-based high density cDNA arrays were developed in Pat Brown's laboratory at Stanford University, USA [1]. cDNA arrays are now a very common choice in academic institutions because they offer a great degree of flexibility in the choice of the arrayed elements. Manufacturing them, however, requires a considerable infrastructure. The process needs liquid handling workstations to work with large collection of cDNA clones and to support the production of the purified probes and, of course, it requires robotics for the production of the

proper microarrays. More recently, several companies (Illumina ►<http://www.illumina.com>), PharmaSeq (►<http://www.pharmseq.com>), and SmartBead Technologies (►<http://www.smartbead.com>) have been producing nonplanar DNA microarrays that allow a higher level of miniturization and very high throughput. The illumina BeadArray™ technology is based on fibre optic strands bundled together. A "microwell" is etched into each fibre core, and this can be filled with oligonucleotide-functionalized microspheres, to which probe sequences are attached [2]. This technology offers a number of advantages over earlier arrays. The combination of the smallest individual feature sizes currently available and a high packing density, allows a greater number of probes and so a higher throughput. The small size of the array also allows smaller volumes to be examined, and improves detection.

The first step in designing a microarray is the choice of the cDNA clones or the determination of the oligonucleotide sequence which will represent the genes of interest. In both cases it is essential to use highly specific sequences to minimize cross-hybridization with related genes. An excellent source of gene information to design microarray probes are public domain expressed sequence databases such as Unigene, Genebank, dbEST. The Unigene database ([▶www.ncbi.nlm.nih.gov/UniGene/](http://www.ncbi.nlm.nih.gov/UniGene/)) is a collection of sequence clusters which represents the large majority of transcribed genes in a variety of species. It is also an excellent source of general information because it provides links to functional and genetics data, to scientific literature and indicates the source of the physical cDNA clones (Fig. 1a–c).

Because of the high gene coverage achievable with this technology it is possible to design arrays which cover the entire transcriptional capacity of an organism. For some species genome-wide arrays are already available. These are mainly bacteria and yeast open reading frame (ORF) based arrays. Human arrays covering the majority of the expressed genes are also available but it is still impractical to design human arrays that cover all the possible splicing variants encoded in the genome. With the amount of effort dedicated to the annotation of the human genome it is expected that more comprehensive arrays will be soon available.

The target: In the last few years more methods to synthesize a labeled target have been introduced. The new methods are still based on a reverse transcription reaction but diverge in the way the fluorescent dye is incorporated in the target. For example, Genechip™ technology developed by Affymetrix uses a reverse transcription step followed by an RNA in vitro transcription reaction to produce a biotinylated target. The detection of the target, after hybridization, is achieved with a streptavidin-bound single fluorescent dye. Targets derived from different biological samples are always hybridized to multiple arrays. In the case of spotted arrays, two or more samples (typically experimental and control sample) can be compared on a single array. This is possible because the targets are labeled by direct incorporation of different fluorescent dyes (e.g., Cy3 and Cy5). After labeling, the targets are mixed and hybridized to the same array, resulting in competitive binding of the target to the sequences on the array (Fig. 1d,e). With both methods, after hybridization and washing, the slides are scanned using one (Affymetrix) or multiple (spotted arrays) wavelengths corresponding to the dyes used (Fig. 1f).

A relatively large amount of RNA needs to be labeled with fluorescent dyes in order to obtain satisfactory results (20–100 µg of total RNA or 1–2 µg of mRNA). These requirements could make microarrays incompatible with the very limited RNA yields obtained from some

type of biopsy or microdissected samples. To bypass these limitations several amplification methods have been developed. PCR-based amplification methods are quite straightforward but they introduce a bias in the gene representation due to the exponential amplification of the original mRNA molecules. Slightly more complex protocols are based on linear in vitro transcription. Although more reliable than PCR-based methods, they also introduce some bias. An alternative strategy to amplification methods has been developed by Genisphere Inc. ([▶www.genisphere.com](http://www.genisphere.com)). The target is synthesized using a reverse transcriptase reaction that introduces a sequence tag at the 3' end of every cDNA. A large agglomerate of oligonucleotides containing several hundreds of fluorescent dyes (dendromere) and containing a tag complementary to the cDNA tag is hybridized to the target to form a labeled cDNA which can be hybridized to the microarray. The large number of fluorophores attached to each cDNA molecule results in a 200-fold signal enhancement.

Amplification based methods can be used with as little as nanograms of total RNA (corresponding to <1000 cells) whereas the dendromer-based methods need at least one microgram of total RNA.

With the decrease in costs associated to synthesis of large collections of oligonucleotides, oligo-based arrays are becoming the technology of choice. Most established model organisms have oligo arrays (e.g., Affymetrix) readily available but this is not the case for less well studied organisms. cDNA arrays are still an important choice for non-model species [2].

Data analysis: The rapid spread of microarray gene expression profiling has created an unprecedented situation in biology. An incredibly large amount of genome-wide gene expression data has been rapidly produced creating the need for appropriate data analysis tools. Many applications have been produced in academic groups and many others are available through commercial providers. Briefly, there are four levels of analysis which have been applied to gene expression profiling. The first uses statistical tests to identify genes differentially expressed between two or more samples. The second utilizes data reduction techniques to simplify the complexity of the dataset by identifying clusters of genes with similar expression profile across different experimental conditions. One of the most common data reduction methods is called Cluster analysis. This method is based on a correlation measure that assigns a high score to genes that have very similar expression profiles. Using this matrix a roadmap displaying the degree of similarity between the genes in the array is built and visualized in a form of a hierarchical tree. The most correlated genes being on very close branches. Even the simple visual inspection of the tree is usually sufficient to identify meaningful associations. The third level involves the use of statistical modeling to link the molecular state of a cell

or tissue with its physiology. A statistical model may be a classifier to identify genes predictive of sample classes (for example tumor vs. normal tissue) or to the expression of an interesting biomarker. An example of the former is the *nearest centroid* classifier. In this method the molecular profile of an unknown sample is compared to the average profile of each class. The sample is then assigned to the class that it is closest to. In statistical modeling it is important to assess the accuracy of the model. A classic approach to this problem is the *holdout* method. In this approach, the correct class labels are known and the data is split into a training and test set. A model is constructed using the training set. The test set provides a group of samples previously unseen by the model, and these can then be used to test if the model correctly predicts their class membership on an independent population. It is desirable to construct such models using a small number of the total genes in the data set, and so a method of gene selection is required. Genes may be tested individually (*univariate gene selection*), or in association (*multivariate* selection strategies). The fourth level of analysis uses reverse engineering algorithms to infer the structure of transcriptional pathways within the cell. This approach has been demonstrated to be very powerful and recently has shown to be robust to extensive experimental verification.

Other Applications

Genotyping of Human Single nucleotide polymorphism (SNPs): The human genome project has produced large amounts of genome sequence and polymorphism data which is extremely useful to investigate the genetic bases of human diseases. Among these, single nucleotide polymorphisms (SNPs) are the most frequent variant in the human genome since they occur once every 1 kb of genomic DNA. Identification of disease genes, however, requires linkage and association analysis of thousands of SNPs in thousands of individuals. This is an impossible task without a high throughput genotyping method. Microarray-based sequencing have been successfully used to genotype SNPs in human populations [3].

Microarray analysis of Chromatin Immunoprecipitation (Chip-on-chip): Protein–DNA interactions are key in the regulation of transcription, DNA replication, DNA repair, DNA recombination, and genome packaging. Discovering the location of these interactions at a genome level is therefore crucial in order to fully understand the mechanisms involved. The ChIP procedure starts by crosslinking growing cells using formaldehyde. Formaldehyde crosslinking causes DNA-bound proteins to be covalently attached to their DNA site. The crosslinked cell extracts are then immunoprecipitated with antibodies against the protein of interest. After this the DNA is purified, the relative amounts of different genomic regions can be amplified, labeled, and hybridized to a microarray. Regions bound in vivo by the

protein of interest will be represented by probes on the microarray that have a relatively high ratio of sample/control binding. In this way it is possible to determine the association of a protein across the whole genome (for a complete overview of this approach see [2]).

Protein arrays: The amount of steady-state RNA in a cell is not always reflecting the biological activity of an expressed gene. It is the protein translated from the RNA pool that exerts the function. It has been proven that mRNA and protein abundance are often not correlated. It follows that information about protein levels and their interactions are essential to understand the biological mechanisms. Microarray technology has been used to increase the throughput of classical protein detection methods and recently to study protein–protein interactions. Protein arrays are more complex than DNA arrays. The major issue is that proteins must bound to the slide while retaining their correct folding. In spite of these difficulties the field is rapidly expanding. Many companies are investing in developing and improving the technology. An excellent example of protein arrays and their applications is described by MacBeath et al. [4]. The authors have deposited 10,000 protein spots on a glass microscope slide and demonstrated that their arrays could detect protein–protein interactions, reveal interactions between enzymes and their substrates, and detect small molecules–protein interactions. Protein microarrays consisting of either printed antibodies or printed protein antigens are used to monitor, respectively, protein levels or antibody levels. With the effort now being concentrated in developing antibodies for the whole of the human proteome, the broad use of protein microarrays is not far in the future.

Pharmacological Relevance

The ability of microarray technology to describe the behavior of thousands of molecular markers is well suited to identify and characterize the effect of a drug in complex biological systems and provide insights into the mechanism of action of the drug, its toxicity, and the molecular basis of drug resistance. Below are some examples of the use of microarray technology in the pharmacology/drug discovery area.

Sensitivity to drugs: It is known that the expression of certain genes can influence the response to drugs. Misexpression or mutations in these genes can sometimes be responsible for treatment failure. This problem is particularly severe in anticancer therapy where resistant cell clones can expand very rapidly and generate secondary tumors which are completely unresponsive to therapy. A general method for the rapid identification of genes influencing drug sensitivity is therefore crucial for the development of future therapies. Uwe Scherf at the National Cancer Institute (NCI) in Bethesda (USA) has used a gene expression profiling based approach to identify genes responsible for influencing the response of

a panel of 60 human cancer cell lines (NCI-60 panel) to a number of anticancer drugs. The data were analyzed using a clustering-based method that relates gene expression in the tumor cell before drug treatment to its capacity to respond to the drug [5]. Interestingly, the authors identified a large number of significant correlations, many of them related to known mechanisms of drug resistance.

Drug mode of action studies (MOA): Conventional molecular and biochemical approaches to MOA studies are usually time consuming and resource demanding. A group of researchers from Rosetta Inpharmatica has recently developed a gene expression profiling based method to identify drug targets that may significantly speed up investigation in this area.

The principle is simple. The expression fingerprint of yeast cells treated with a compound with unknown MOA is compared with a reference set of transcriptional profiles representing 300 *Saccharomyces cerevisiae* gene knock out mutants. The mutant with the closest expression fingerprint to the compound-treated cells will most likely be mutated in a gene related to the drug target. Sequence homology to the identified yeast gene may then reveal the potential drug target in more broadly pharmaceutically relevant species (i.e., mouse, rat, and man). Interestingly the analysis of the expression profiles of yeast cells treated with the anesthetic drug dyclonine identified the human neuro-active sigma factor as a potential target. Although there are potential complications in a broader application of gene expression profiling to the analysis of a drug mode of action directly in mammalian cells several more studies have been reported [5].

Toxicology: Many companies are known to use gene expression profiling to assess the potential toxicity of lead compounds. This approach may require a database of reference compounds with known pharmacological and toxicological properties. Lead compounds can be compared to the database to predict compound-related or mechanism-related toxicity [5].

Pharmacogenomics: This rapidly expanding field examines the genetic basis for individual variations in response to therapeutics. Mutations in certain genes involved in drug metabolism make some populations unresponsive to a certain drug. Microarray technology can be used to genotype patients and predict their response to therapy. In this context pharmacogenetics promises to increase the success of pharmaceutical research by developing individualized medicines tailored to patients' genotypes [5].

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Microbial Resistance to Drugs

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Synonyms

Antibiotic resistance

Definition

A bacterial isolate is called resistant if it grows in a relatively high concentration of a specific antibacterial drug. Thus, it is insensitive. The sensitivity is measured with standardized methods as the ►**minimal inhibitory concentration** (MIC). Guidelines from institutions like the CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS) and DIN (Deutsches Institut für Normung e.V.) define specific breakpoints above which MIC an isolate is regarded as resistant. This breakpoint usually is derived from microbiological and clinical experience and resistance is associated with a high likelihood of therapeutic failure (Fig. 1).

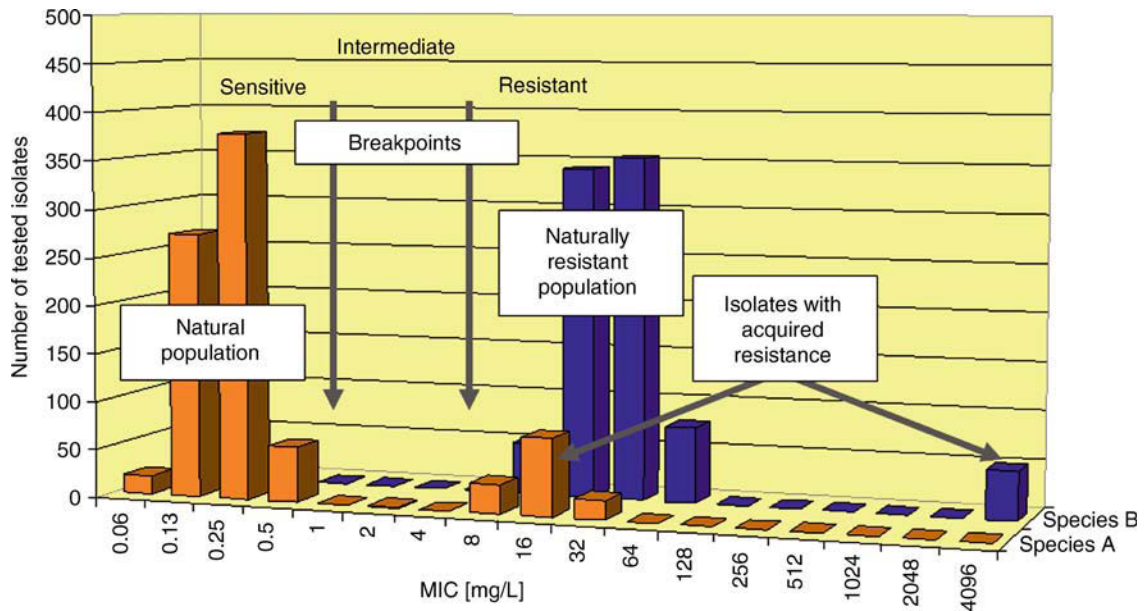
Basic Mechanisms

Bacteria can develop resistance to antimicrobial agents as a result of mutational changes in the ►**chromosome** or via the acquisition of genetic material (resistance genes carried on ►**plasmids** or ►**transposons** or the recombination of foreign DNA into the chromosome) (Fig. 2).

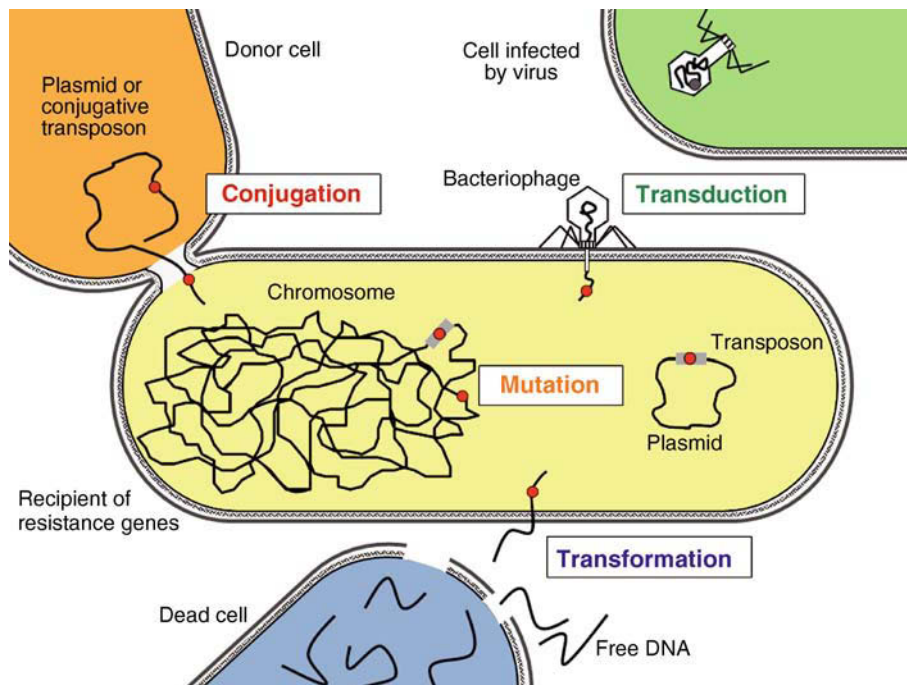
The basic biochemical mechanisms leading to bacterial resistance can be classified into three different categories.

Antibiotic Inactivation

A common means that causes resistance is the inactivation of the antibiotic before it reaches the target site. Antibiotics can be either enzymatically cleaved or modified. In both cases the antibiotic loses its capacity to bind to its target.



Microbial Resistance to Drugs. Figure 1 MIC-distribution showing the number of isolates of one species with a certain MIC. Some bacterial species are naturally resistant (intrinsic resistance) to drugs because their natural MIC is above the breakpoint. Naturally sensitive isolates as well as naturally resistant ones can acquire resistance and with that increase their MIC (acquired or secondary resistance).



Microbial Resistance to Drugs. Figure 2 Bacterial cells can obtain resistance genes in three ways: (i) Bacteria receive a plasmid or a conjugative transposon carrying resistance genes by a mechanism that involves direct cell-to-cell contact between donor and recipient cell (conjugation). (ii) A bacteriophage infects a bacterium carrying a resistance gene and transfers the gene to the recipient cell where it can be incorporated into the genome (transduction). (iii) Free DNA from dead cells in the vicinity of the recipient cell is taken up and integrated into the chromosome (transformation).

Enzymatic Cleavage

β -Lactamases are enzymes that hydrolyze the β -lactam ring of β -lactam antibiotics (penicillins, cephalosporins, monobactams and carbapenems). They are the most common cause of β -lactam resistance. Most enzymes use a serine residue in the active site that attacks the β -lactam-amid carbonyl group. The covalently formed acylester is then hydrolyzed to reactivate the β -lactamase and liberates the inactivated antibiotic. Metallo β -lactamases use Zn(II) bound water for hydrolysis of the β -lactam bond. β -Lactamases constitute a heterogeneous group of enzymes with differences in molecular structures, in substrate preferences and in the genetic localizations of the encoding gene (Table 1).

Another group of antibiotics that can be inactivated by hydrolysis are 14- and 15- membered macrolides [2]. Esterases cleave the lactone ring. The plasmid encoded *ere* genes are found in members of the *Enterobacteriaceae* and increase the intrinsic resistance. Furthermore, these esterases can also be found in some isolates of erythromycin resistant staphylococci.

Resistance to streptogramin type B antibiotics can be mediated in staphylococci and enterococci by plasmids carrying a *vgb* gene [2]. The Vgb enzyme is a lyase that linearizes the cyclic hexadepsipeptide by cleavage of the ester bond via an elimination reaction.

Chemical Modification

Several bacterial enzymes can modify antibiotics to inactive derivatives (Table 2).

Enzymatic modification is the most important mechanism of aminoglycoside resistance in gram-positive and gram-negative bacteria. There are three different types of enzymatic activities. *N*-acetyltransferases (AAC) use acetyl-CoA as a donor to modify amino groups in the aminoglycoside. ATP dependent *O*-adenyltransferases (ANT) and ATP dependent *O*-phosphoryltransferases (APH) modify hydroxyl residues. Numerous enzymes have been described. The nomenclature includes the regiospecificity of the group transfer (e.g. 3"), a roman numbering distinguishes between substrate specificities and an alphabetic prefix differentiates different genes. The localization of the genes can be chromosomal but is usually plasmidic and can often be found on transposable elements [3].

It has been shown that an AAC enzyme variant (AAC (6')-Ib-cr, cr for ciprofloxacin resistance) found in various *Enterobacteriaceae* is capable of *N*-acetylating fluoroquinolones with an unmodified piperazinyl substituent at the amino nitrogen. Although the increase in MIC is low, this plasmid encoded quinolone resistance determinant can augment further development of clinically relevant resistance.

Acetyl-CoA is also utilized as a cofactor to modify chloramphenicol by *O*-acetyltransferases (CATs). These enzymes have been found in many different bacterial genera and are usually plasmid encoded in clinical isolates. Furthermore, streptogramin type A antibiotics are acetylated by Vat enzymes that occur on plasmids in staphylococci and enterococci.

Microbial Resistance to Drugs. Table 1 Classification of β -lactamases [1]

Molecular class	Functional mechanism	Examples of enzymes ^a	Types ^b
Class A	Serine β -lactamases	SHV-1 penicillinase in <i>K. pneumoniae</i> , and Koxy with activity against certain third generation cephalosporins in <i>K. oxytoca</i>	BlaZ staphylococcal penicillinase; TEM, SHV, VEB, PER and CTX-M penicillinases and ESBLs (β -lactamases with activity against third generation cephalosporins and aztreonam) KPC, IMI/NMC and SME carbapenemases
Class B	Metallo β -lactamases	L1 enzyme of <i>S. maltophilia</i> ; enzyme of <i>Aeromonas</i> spp.; CcrA enzyme found in 1–3% of <i>B. fragilis</i> isolates. All enzymes are carbapenemases	IMP, VIM and SPM type carbapenemases
Class C	Serine β -lactamases	AmpC enzymes of <i>E. coli</i> , <i>Shigella</i> spp., <i>Enterobacter</i> spp., <i>C. freundii</i> , <i>M. morgani</i> , <i>Providencia</i> spp. and <i>Serratia</i> spp. cephalosporinases with wide spectrum of activity	CMY, LAT, BIL, MOX, ACC, FOX and DHA types. All genes are <i>ampC</i> genes that have been mobilized by transfer to plasmid DNA.
Class D	Serine β -lactamases	OXA enzymes (oxacillinases) of <i>Acinetobacter</i> spp. and some <i>Aeromonas</i> spp. Some OXA enzymes are carbapenemases	Most OXA types are chromosomal

^aEnzymes that are normally chromosomal and ubiquitous. Some are produced at low levels; mutations can lead to high-level production.

^bTypes that are plasmid- or transposon-mediated, generally produced at high levels.

Microbial Resistance to Drugs. Table 2 Antibiotic modifying enzymes [2, 3]

Enzymes	Substrates	Host	Gene localization
Acetyltransferases			
AAC	Aminoglycosides, fluoroquinolones	Gram-negative and gram-positive bacteria	Plasmid, transposon, chromosome
Vat	Streptogramin A	Staphylococci, enterococci	Plasmid
CAT	Chloramphenicol	Gram-negative and gram-positive bacteria	Plasmid, chromosome, transposon
Adenyltransferases			
ANT	Aminoglycosides	Gram-negative and gram-positive bacteria	Plasmid, transposon
Lnu	Lincomycin	Staphylococci, <i>E. faecium</i>	Plasmid
Arr	Rifampicin	Gram-negative bacteria	Plasmid
Phosphotransferases			
APH	Aminoglycosides	Gram-negative and gram-positive bacteria	Plasmid, transposon
Mph	Macrolides	<i>E. coli</i> , <i>S. aureus</i>	Plasmid
Glutathionetransferase			
Fos	Fosfomycin	Gram-negative and gram-positive bacteria	Plasmid

Nucleotidylation – the addition of adenylate-residues by Lnu enzymes – can also be the cause of resistance to lincosamide antibiotics in staphylococci and enterococci. A plasmid encoded ADP-ribosylating transferase (Arr-2) that leads to rifampicin resistance has been detected in various *Enterobacteriaceae* as well as in *Pseudomonas aeruginosa*.

O-phosphotransferases that modify macrolides are produced by highly macrolide resistant *E. coli* isolates. However, these enzymes have no clinical importance for macrolide resistance in gram-positive bacteria, and gram-negative ones are regarded as naturally resistant [2].

A minor percentage of fosfomycin resistance in gram-positive and gram-negative species is due to plasmids carrying a *fos* gene. The Fos protein, a glutathione-S-transferase, catalyzes the opening of fosfomycin followed by the addition of the tripeptide glutathione to the antibiotic.

Prevention of Access to the Target

The second general mechanism to cause resistance to antibacterial agents is to prevent the drug from reaching its target site. This is either achieved by altered rates of entry (reduced uptake) or by the active removal of the drug (active efflux) [4].

Reduced Uptake

The outer membrane of gram-negative bacteria is a permeability barrier that allows the passive diffusion of small hydrophilic antibiotics only through aqueous channels, the porins. Drugs larger than 800 Da are

excluded. Mutational changes that lead to a reduction in the number of porins or the size of their diameter slow down the penetration process. In combination with a second contributor, e.g. a β -lactamase, porin changes can have a pronounced effect on the MIC. Accordingly, carbapenem resistance has been described in clinical *Enterobacter* and *Citrobacter* isolates due to the loss of a major porin protein combined with a high-level production of an AmpC β -lactamase that is normally not able to confer resistance to carbapenems.

Furthermore, if the antibiotic passes membranes through a specific port of entry, its mutational loss leads to resistance. The lack of the outer membrane protein OprD in *P. aeruginosa* causes resistance to the β -lactam antibiotic imipenem. Fosfomycin passes the cytoplasmic membrane via an L- α -glycerol phosphate permease. This transport system is not essential for bacterial growth and therefore mutants with a reduced expression are frequently selected under therapy.

Active Efflux

Bacterial resistance can be caused by actively pumping antibiotics out of the cell and therefore decreasing the concentration at the target site. Drug efflux systems in bacteria are classified into four major groups based on their sequence homologies and functional similarities (Table 3).

ATP-binding cassette (ABC) transporters are efflux pumps that derive the energy needed for drug extrusion from the hydrolysis of ATP. Bacterial ABC antibiotic efflux transporter encoded on plasmids is a significant

Microbial Resistance to Drugs. Table 3 Examples of frequent bacterial efflux systems [4]

System	Substrates	Species	Gene location
ABC			
MsrA	14-, 15-Membered macrolides, streptogramin type B	Staphylococci	Plasmid
Vga, Vga(B)	Streptogramin type A	<i>S. aureus</i>	Plasmid
SMR			
Smr (QacC)	Quarternary ammonium compounds	<i>S. aureus</i>	Plasmid
MFS			
NorA	Fluoroquinolones	<i>S. aureus</i>	Chromosome
TetK	Tetracycline	<i>S. aureus</i>	Plasmid
QacA	Quarternary ammonium compounds	<i>S. aureus</i>	Plasmid
TetA	Tetracycline	<i>E. coli</i>	Plasmid
MefA	14-, 15-Membered macrolides	<i>S. pyogenes</i>	Chromosome
MefE	14-, 15-Membered macrolides	<i>S. pneumoniae</i>	Chromosome
RND			
AcrAB-TolC	Tetracycline, fluoroquinolones, chloramphenicol, β -lactams except imipenem, novobiocin, erythromycin, fusidic acid, rifampicin	<i>E. coli</i>	Chromosome
MexAB-OprM	Tetracycline, fluoroquinolones, chloramphenicol, β -lactams except imipenem, novobiocin, erythromycin, fusidic acid, rifampicin, trimethoprim, sulfamethoxazol	<i>P. aeruginosa</i>	Chromosome

contributor to the acquired resistance of staphylococci to macrolide and streptogramin antibiotics. The energy for the efflux mediated by the other three groups of bacterial transporters is provided by the proton-motive-force. Transporters of the SMR family (*Staphylococcus* multi-drug resistance) are plasmid encoded small cytoplasmic membrane proteins. The Smr transporter of *Staphylococcus aureus* pumps out quarternary ammonium compounds (e.g. benzalkonium chloride) and therefore confers resistance to these disinfectants. Within the MFS (major facilitator superfamily) group there are several pumps acting on different classes of antibiotics (Table 3). The respective genes are found on conjugative and non-[conjugative plasmids](#) or within the chromosome. They can be part of [conjugative transposons](#) as seen with the *mef* genes. Transporters of the RND (resistance/nodulation/division) family are chromosomally encoded. These systems are typically tripartite. A pump protein is located in the cytoplasmic membrane and an “outer membrane protein” forms a transperiplasmic tunnel and a channel through the outer membrane. The contact between them is established by a “membrane fusion protein”. The efflux pumps of *P. aeruginosa* contribute significantly to the natural resistance of this species to a wide range of antibiotics. Furthermore, they can confer high levels of resistance when overexpressed as a result of mutations within regulatory genes.

Altered Target

A third general mechanism of bacterial resistance is to obtain an unsusceptible target. The affinity of the antibiotic to the target is diminished without impairing the physiological function of the target considerably. This can be either achieved by altering the usual target or by the acquisition of a new unsusceptible target [5].

Alteration of the Usual Target

One general mechanism is to exchange residues within the target molecule.

Resistance to fluoroquinolones is frequently mediated by amino acid changes within specific domains of the target proteins gyrase and topoisomerase IV. Mutations occur at hotspots in a quinolone resistance-determining region (QRDR) of the genes *gyrA* (coding for subunit A of gyrase) and *parC/grlA* coding for subunit A of topoisomerase IV.

One amino acid substitution within a specific region in the β -subunit of the RNA-polymerase, the target molecule of rifampicin, is necessary to establish high-level resistance to this antibiotic.

Macrolide, lincosamide and streptogramin B resistance (MLS_B phenotype) can be linked to specific nucleotide changes within the 23S rRNA of the large ribosomal subunit, mainly at position A2058 or neighbouring bases (*E. coli* numbering). This is the

major mechanism of macrolide resistance in *Helicobacter pylori*, a species that does not possess multiple but only two *rrn* operons.

The acquisition of a *vanA* gene cluster carried on a transposon is the major mechanism to confer glycopeptide resistance in enterococci (vancomycin resistant enterococci, VRE). Recently, few isolated cases of *vanA* gene clusters in clinical *S. aureus* isolates (VRSA) have also been reported. A coordinated expression of several *van* genes leads to the synthesis of modified peptidoglycan precursors: The D-alanyl-D-alanine moiety, the target of glycopeptides, is replaced by D-alanyl-D-lactate, which has a decreased affinity for vancomycin and teicoplanin.

A similar complex mechanism is responsible for the penicillin resistance of pathogenic *Neisseria* and *Streptococcus pneumoniae*. These bacteria are naturally competent, meaning they are able to take up free DNA. They alter the targets for penicillin, the penicillin binding proteins (PBPs), by recombining parts of their PBP genes with homologous DNA from related species. The resulting mosaic genes then encode PBPs with low affinity to penicillin.

A second mechanism that can be grouped into the category of alteration of the usual target is the overproduction of the target.

The enterococcal penicillin-binding-protein PBP5 binds penicillin with low affinity. The overproduction of this PBP is able to compensate the loss of the others which are inhibited by the drug.

Overproduction of the chromosomal genes for the dihydrofolate reductase (DHFR) and the dihydropteroate synthase (DHPS) leads to a decreased susceptibility to trimethoprim and sulfamethoxazol, respectively. This is thought to be the effect of titrating out the antibiotics. However, clinically significant resistance is always associated with amino acid changes within the target enzymes leading to a decreased affinity of the antibiotics.

A third common means to alter the target is its modification. A widespread mechanism, with high clinical relevance in staphylococci and streptococci, leading to a MLS_B phenotype encoded by *erm* genes, is mainly found on conjugative and non-conjugative transposons. The Erm enzymes add one or two methyl groups to the adenine residue A2058 of the 23S ribosomal RNA. This phenotype is either constitutive or inducible by macrolide antibiotics via a translational attenuation mechanism.

Resistance to tetracyclines is often caused by the acquisition of genes (e.g. *tetO* and *tetM*) coding for so-called ribosome protection proteins. These proteins bind to the ribosome and protect them from tetracycline action.

The enzymes gyrase and topoisomerase IV can be protected from fluoroquinolone inhibition by a small protein belonging to the pentapeptide-repeat family of

proteins, termed QNR. The *qnr* gene is plasmid encoded and its acquisition leads to a reduced susceptibility to fluoroquinolones (Hooper). Although *qnr* alone does not lead to clinical resistance, it can facilitate the selection of higher-level resistance.

Acquisition of New Unsusceptible Targets

Methicillin resistant *S. aureus* (MRSA) isolates produce an acquired new PBP with low affinity to almost all β -lactam antibiotics. It allows functional cell wall synthesis even if the normally occurring PBPs are inhibited. This PBP2a (or PBP2') is encoded by the *mecA* gene that is part of a region of foreign DNA (*mec* region) that has been integrated into the staphylococcal chromosome. MRSA is a major problem as these isolates also tend to display a multi-resistance phenotype to other classes of antibiotics.

Resistance to trimethoprim can be due to the acquisition of plasmid encoded non-allelic variants of the chromosomal DHFR enzyme that are antibiotic unsusceptible. The genes may be part of transposons that then insert into the chromosome. For instance, in gram-negative bacteria the most widespread gene is *dhfrI* on transposon Tn7.

Accessory DHPS enzymes confer resistance to sulfonamides. Two different types encoded by the genes *sulI* (located on transposons) and *sulII* (located on plasmids) have been described. These resistance determinants are often genetically linked to trimethoprim resistance genes. Therefore, the combination of sulfonamide antibiotics with trimethoprim does not prevent resistance selection.

Drug resistance in the defined sense, however, is not always the reason for treatment failures. The formation of biofilms may be as well regarded as a resistance mechanism. Cells within such a film withstand the antibiotic treatment. Some antibiotics (e.g. the aminoglycoside tobramycin) penetrate only slowly into the film. A further explanation is the existence of cells living in a non-growing, protected phenotypic state.

Furthermore, the inability of the drug to reach the focus of the infection or to reach bacteria with intracellular location may be a common reason for the failure of antibiotic treatment.

Pharmacological Relevance

The presence of a specific resistance mechanism in a bacterial isolate does not necessarily implicate that this isolate is resistant in clinical terms. However, an isolate expressing a resistance mechanism possibly will be eliminated less easily as compared to a susceptible one. In clinical practice, resistance mechanisms will be rarely identified. Usually the sensitivity of isolates will be determined and reported to the clinician by using the interpretive criteria which is sensitive, intermediate or resistant. Some bacteria harbouring resistance

mechanisms may show up as sensitive in the standard test, however, therapy will probably fail.

- **β -Lactam Antibiotics**
- **Quinolones**
- **Ribosomal Protein Synthesis Inhibitors**

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Microfilaments

- **Cytoskeleton**

Microtubule

A microtubule is a hollow tube of 25 nm diameter formed by 13 protofilaments. Each protofilament consists of polymerized α - and β -tubulin heterodimers. Microtubules are polarized and have a plus and a minus end.

- **Cytoskeleton**

Microtubule Associated Proteins

Microtubule associated proteins (MAPs) are attached to microtubules in vivo and play a role in their nucleation, growth, shrinkage, stabilization and motion.

- **Cytoskeleton**

Mineralcorticoids

The main endogenous mineralocorticoid is aldosterone, which is mainly produced by the outer layer of the adrenal medulla, the zona glomerulosa. Aldosterone, like other steroids, binds to a specific intracellular (nuclear) receptor, the mineralocorticoid receptor (MR). Its main action is to increase sodium reabsorption by an action on the distal tubules in the kidney, which is accompanied by an increased excretion of potassium and hydrogen ions.

- **Glucocorticoid Receptors**

Mineralocorticoid Receptor (MR)

- **Glucocorticoid Receptors**

Minimal Inhibitory Concentration

The minimal inhibitory concentration (MIC) is the concentration which is able to prevent 10^5 cells/mL from growing up to a visible density ($\sim 10^8$ cells/mL) under standardized conditions.

- **Microbial Resistance to Drugs**

MinK

This minimal K^+ channel (MinK) encoded by *KCNE1* consists of 130 amino acid residues and has a single transmembrane segment. A slowly activating K^+ current-induced MinK cRNA is expressed in *Xenopus* oocytes. Coexpression of KvLQT1 with MinK induced a current that has characteristics similar to cardiac slowly activating delayed-rectifier K^+ current, IK_S , in contrast to IK_R that has relative fast activation and is composed of hERG/MiRP1.

- **K^+ Channels**

MirP Subunits

MirP subunits are relatives of MinK subunits. The abbreviation means MinK related Protein. So far, four MirP subunits are known. The corresponding human genes are KCNE2 to KCNE5. MirP1 (KCNE2) may associate with HERG channels. Mutations in MirP1 have been associated with certain forms of the long QT syndrome.

► K⁺ Channels

Mitochondrial Permeability Transition Pore

Mitochondrial permeability transition involves the opening of a larger channel in the inner mitochondrial membrane leading to free radical generation, release of calcium into the cytosol and caspase activation. These alterations in mitochondrial permeability lead eventually to disruption of the respiratory chain and depletion of ATP. This in turn leads to release of soluble intramitochondrial membrane proteins such as cytochrome C and apoptosis-inducing factor, which results in apoptosis.

► Apoptosis

Mitogen

Substances that cause cells, particularly lymphocytes, to undergo cell division. Mitogens are also referred to as polyclonal activators, since they stimulate proliferation of lymphocytes irrespective of their clonal origin. The best known mitogens are phytohemagglutinines isolated from certain plants.

- Interferons
- Growth Factors

Mitogen-activated Protein Kinase

- MAPK
- MAP Kinase Cascades
- Glucocorticoids

Mitosis

The phase of the cell cycle where the sister chromatids are separated and distributed onto two daughter nuclei. First, upon entry into mitosis the chromosomes are condensed followed by the breakdown of the nuclear envelope (prophase). The two centrosomes are separated and induce the formation of the mitotic spindle. Then, the chromosomes are captured by the spindle and aligned on the metaphase plate (metaphase). The sister chromatids are separated and pulled to the poles of the spindle (anaphase). In telophase, two new nuclei are formed around the separated chromatids.

► Cell Cycle Control

Mixed Agonists/Antagonists

► Selective Sex Steroid Receptor Modulators

Mixed Function Oxygenase

► P450 Mono-oxygenase System

MLCK

► Myosin Light Chain Kinase

MLCP

► Myosin Phosphatase

MMPs

► Matrix Metalloproteinases

Molecular Cancer Therapeutics

► Targeted Cancer Therapy

Molecular Dynamics

► Molecular Modeling

Molecular Dynamics Simulation

The principle of the molecular dynamics simulation approach is the movement of atoms under the action of a force field.

This approach starts with an initial low-energy structure and the integrated Newton's equation of motion is solved for all atoms simultaneously. Often the protein is placed in a box, which is filled up with solute molecules, e.g., water. To avoid effects at the edges a trick is used, the so-called periodic boundary condition. At the beginning, a starting velocity is assigned to every atom. The velocities are selected to couple the system on a certain temperature bath e.g., 310 K. For every atom, the force which is acting on this particle by the surrounding atoms is calculated. With a given step, usually 1 femtosecond, the next position is calculated over time to obtain classical atomic trajectories, which define conformational motions.

► Molecular Modeling

Molecular Modeling

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Synonyms

Biocomputing; Molecular simulation; Molecular dynamics; Structural bioinformatics

Definition

Molecular modeling itself can be simply described as the computer-assisted calculation, modulation, and visualization of realistic 3D-molecular structures and their physical–chemical properties using ► [force fields/molecular mechanics](#).

Moreover, molecular modeling is one key method of a wide range of computer-assisted methods to analyze and predict relationships between protein sequence, 3D-molecular structure, and biological function (sequence–structure–function relationships). In molecular pharmacology these methods focus predominantly on analysis of interactions between different proteins, and between ligands (hormones, drugs) and proteins as well gaining information at the amino acid and even to atomic level.

Description

The constantly increasing amount of data coming from high throughput experimental methods, from genome sequences, from functional- and ► [structural genomics](#) has given a rise to a need for computer-assisted methods to elucidate sequence–structure–function relationships.

Sequence Similarity

Protein sequences encoded by genome sequences encode biological functions.

There are two different dimensions, breadth and depth, used to reveal sequence–structure–functional relationships by computational methods [1].

The aim of the first dimension breadth is to reveal sequence–function relationships by comparing protein sequences by sequence similarity. Simple bioinformatic algorithms can be used to compare a pair of related proteins or for sequence similarity searches e.g., BLAST (Basic Local Alignment Search Tool). Improved algorithms allow multiple alignments of larger number of proteins and extraction of consensus sequence pattern and sequence profiles or structural templates, which can be related to some functions, see e.g., under ► <http://www.expasy.ch/tools/#similarity>.

The aim of the second dimension depth is to consider protein 3D-structures to uncover structure–function relationships. Starting from the protein sequences, the steps in the depth dimension are structure prediction, homology modeling of protein structures, and the simulation of protein–protein interactions and ligand-complexes.

Protein Structure Predictions

Starting from the protein sequence (primary structure) several algorithms can be used to analyze the primary structure and to predict secondary structural elements like beta-strands, turns, and helices. The first algorithms from Chou and Fasman occurred already in 1978. The latest algorithms find e.g., that predictions of transmembrane

and coiled regions from the primary sequence are possible ([►http://dodo.cpmc.columbia.edu](http://dodo.cpmc.columbia.edu)).

Approaches of de novo predictions, which try to calculate how the structural elements are folded into the 3D-structure (tertiary structure) of complete proteins are nowadays far away from reliable large-scale applications. On the other, hand this topic is under strong development indicated by recent successful results at the contest for structural prediction methods CASP4. With the fast growing number of experimentally solved 3D-structures of protein and new promising approaches like threading tools combined with experimental structural constraints, one can expect more reliable de novo predictions for 3D-protein structures in the future.

Homology Models of Protein Structures

The primary sequence of proteins, with identical function varies within different species by natural mutations of amino acids. With increasing distance in the evolutionary process the number of variations between the sequences of proteins increase.

In the protein structure database PDB ([►http://www.rcsb.org/pdb](http://www.rcsb.org/pdb)), by X-ray crystallography and NMR spectroscopy, experimentally solved 3D-protein structures are available to the public. Homology model building for a query sequence uses protein portions of known 3D-structures as structural templates for proteins with high sequence similarity.

If the sequence of a protein has more than 90% identity to a protein with known experimental 3D-structure, then it is an optimal case to build a homologous structural model based on that structural template. The margins of error for the model and for the experimental method are in similar ranges. The different amino acids have to be mutated virtually. The conformations of the new side chains can be derived either from residues of structurally characterized amino acids in a similar spatial environment or from side chain rotamer libraries for each amino acid type which are stored for different structural environments like beta-strands or alpha-helices.

The discrepancies between homologous protein sequences occur predominantly at the surfaces and in the so-called loop regions. With decreasing sequence identities, insertions and deletions of loop chains or modeling of completely different loop chains are necessary. Segmentation of the loop in overlapping sequence fragments (3–10 residues) and searching for sequence similarity using BLAST or FASTA in the PDB database was proven to be most successful in loop modeling. Those fragments occurring several times in different protein structures with a common backbone conformation, have a high probability of adopting the same backbone conformation also in the query sequence. The overlap almost allows knowledge-based assembly of the fragments to a new loop conformation.

This segmentation strategy is also part of the successful algorithm, Rosetta ([►http://depts.washington.edu/bakerpg/](http://depts.washington.edu/bakerpg/)) for predicting complete folds for new proteins.

Similar residues in the cores of protein structures especially hydrophobic residues at the same positions, are responsible for common folds of homologous proteins. Certain sequence profiles of conserved residue successions have been identified which give rise to a common fold of protein domains. They are organized in the smart database (simple modular architecture research tool) [►http://smart.embl-heidelberg.de](http://smart.embl-heidelberg.de).

Natural mutation of amino acids in the core of a protein can stabilize the same fold with different complementary amino acid types, but they can also cause a different fold of that particular portion. If the sequence identity is lower than 30% it is much more difficult to identify a homologous structure. Other strategies like secondary structure predictions combined with knowledge-based rules about reciprocal exchange of residues are necessary. If there is a reliable assumption for common fold then it is possible to identify intra- and intermolecular interacting residues by search for correlated complementary mutations of residues by correlated mutation analysis, CMA (see e.g., [►http://www.fmp-berlin.de/SSFA](http://www.fmp-berlin.de/SSFA)).

Predicting a likely conformation or fold of a particular region of a protein with less or no sequence similarity to protein structures recorded in the PDB, is the main challenges for homology modeling of proteins.

Sequence conservation is, in general, much weaker than structural conservation. There are proteins, which are clearly not related in sequence but are closely related in 3D-structure and fold, like hemoglobin and myoglobin, which have similar functions. In many proteins, fold elements like 4-helical bundles are repeated. Classifications of known structural folds of proteins are organized in the SCOP or CATH database see e.g., [►http://scop.mrc-lmb.cam.ac.uk/scop/](http://scop.mrc-lmb.cam.ac.uk/scop/).

Taken together, the procedure to build a starting protein structural model for a protein combines similarity searches by sequences and by folds in different 3D-structure databases. Remaining unknown conformations are completed by information resulting from bioinformatic, knowledge-based approaches and overlapping segmentation of sequence fragments.

After the construction phase of a model follows the optimization of the geometrical structure by force fields.

Molecular Simulation

Simulation in general describes calculations with models, where different options and combinations of variables can be quickly played through. Molecular simulations allow the characterization of molecular properties during the motions of the molecular models, over time.

Force field or molecular mechanics calculations of molecular models are energy minimizations. Starting with an energy for an unfavorable molecular geometry the algorithm searches for the next local energy minimum at the energy hypersurface. Starting with different unfavorable geometries can lead to different conformations at other local energy minima. For larger molecular structures and especially for structural models build on templates with lower sequence identity, it is necessary to evaluate the geometrical stability.

Importantly, all biological procedures are operating at a temperature of 310 Kelvin, not at 0 Kelvin as the potential energy is calculated by the force fields. The kinetic energy must also be considered. Molecules and proteins at room temperature change the conformation at least at the surface and in loop region. ► **Molecular dynamics simulation** (MD) is an approach to tackle these kinetic and stability problems.

An approach to overcome the multi minima problem of proteins is ► **simulated annealing** (SA) run. Besides global molecular properties such as structural and thermal motions, functional properties of fast biological reactions can also be studied by MD.

Accuracy and Limitations

Molecular models are only an approximation to reality, but good models can often closely approach reality.

The margin of error of a final structural model depends on the sequence or fold similarity to the starting structural template.

An important measure for quality is the verification by MD or SA of the stability of a molecular model. Other programs (e.g., PROCHECK) can also be used to check the globular geometrical quality of a structure to avoid serious defects in the geometry of proteins.

Even the most elaborate models are worthless if there is no experimental examination at all.

Functional insights based on structural relationships can only rise to the level of hypotheses, and these hypotheses must be tested by direct functional experiments.

The strongest verification for a 3D-protein model comes from the experimental 3D-structure. This is the objective of the Critical Assessment of Techniques for Protein Structure Prediction, CASP (► <http://predictioncenter.org>), where the structural models are made in advance of the experimental structure of a particular protein.

In molecular pharmacology research an indirect proof of a structural model is possible by functional examinations, e.g., by molecular biological experiments. Well-selected site directed mutagenesis and their functional characterization allows confirmation or rejection of a molecular protein model. The process is organized as an iterative procedure, where the biological answer of suggested mutations is used to refine the model. The iteration continues until the model

is consistent with the biological experiments and the functional predictions of mutations are confirmed.

Pharmacological Relevance

In general the relevance of predictions of structure–function relationships based on molecular modeling and structural bioinformatics are threefold. First they can be used to answer the question of which partners (proteins) could interact. Second, predictions generate new hypotheses about binding site, about molecular mechanisms of activation and interaction between two partners, and can lead to new ideas for pharmacological intervention. The third aim is to use the predictions for structure-based drug design.

Common to all three aims is that silico-derived predictions can rationalize experimental efforts either by well-directed very specific molecular biological experiments like site directed mutations or e.g., by reducing the number of compounds to screen experimentally for drug design.

Structure–Function Prediction

From the human genome project it is known, that roughly 30,000 proteins exist in humans. Currently only the 3D-structures of few thousand human proteins or protein domains are known. Structures of membrane-bound proteins are several magnitudes rarer. Beside efforts to solve further structures like structural genomics, there is a challenge for computational approaches to predict structures and function for homologous proteins.

This is eminently necessary for large protein classes with important functions, e.g., the ► **G-protein coupled receptors** (GPCR) where several hundred different human GPCR are known. Out of this large family of seven transmembrane-helix proteins, there is only one structure known jet-rhodopsin. The rhodopsin structure is used as a template for homologous receptor models. Incorporation of further experimental results like scanning accessible cysteines, cross linking, spin labeling, ligand binding, and site directed mutations etc., allowed molecular modeling to predict successfully ligand binding sites and local activation mechanisms of diverse GPCR. In general the inactive conformation of GPCR is constrained by interaction of complementary residues in the interior side of the receptors which can be observed in the rhodopsin structure. Activation mechanisms of homologous GPCRs can be revealed by molecular simulations and characterized by molecular experiments as in the following example.

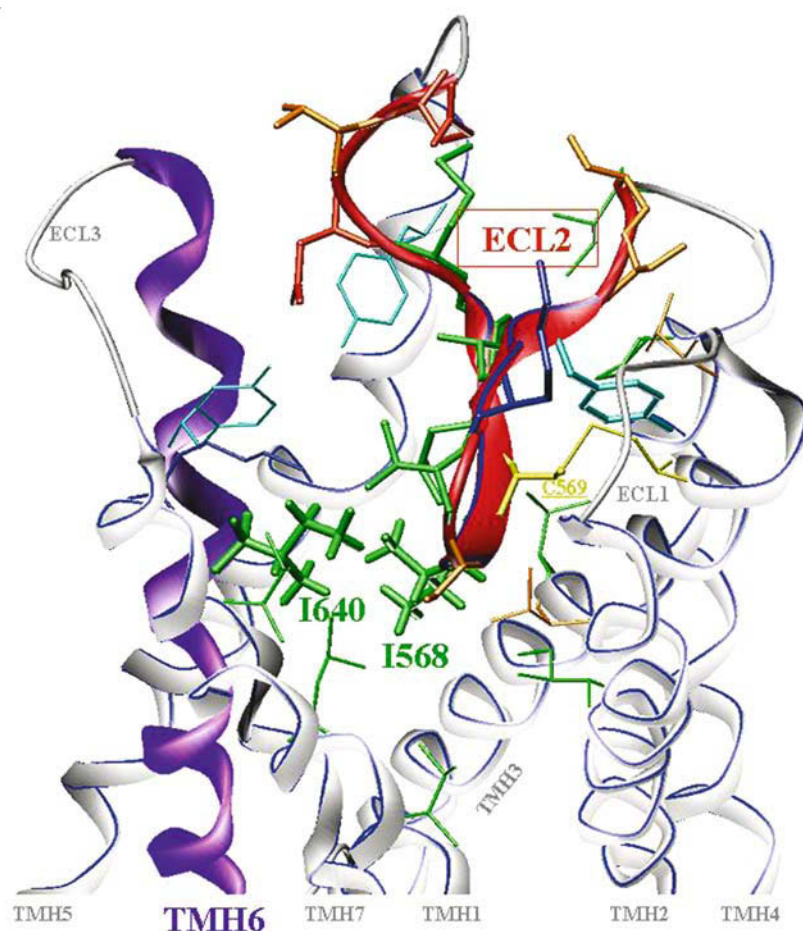
For the thyroid stimulating hormone receptor (TSHR) the homolog of glycoprotein hormone receptors and member of family A of GPCRs, a spatial and functional relationship between extracellular loop 2 (ECL2) and transmembrane helix 6 (TMH6) was suggested by homology modeling. In detail, side chain

interaction between Ile-568 and Ile-640, respectively was predicted to constrain the receptor in a conformation with low basal activity (Fig. 1). Subsequently, it was hypothesized that mutations on either side of the potential counterparts should disrupt, but complementary double mutation should rescue the dynamic interface between ECL2 and TMH6. Indeed, the single mutant Ile640Leu exhibits basal activity lower than wild type, its differently branched and bulkier side chain complements the reduced side chain bulk in Ile568Val, restoring wild type basal activity to the double mutant. This scenario is confirmed by the reciprocal double mutant Ile640Val/Ile568Leu [2]. Disruption of this critical interface between TMH6

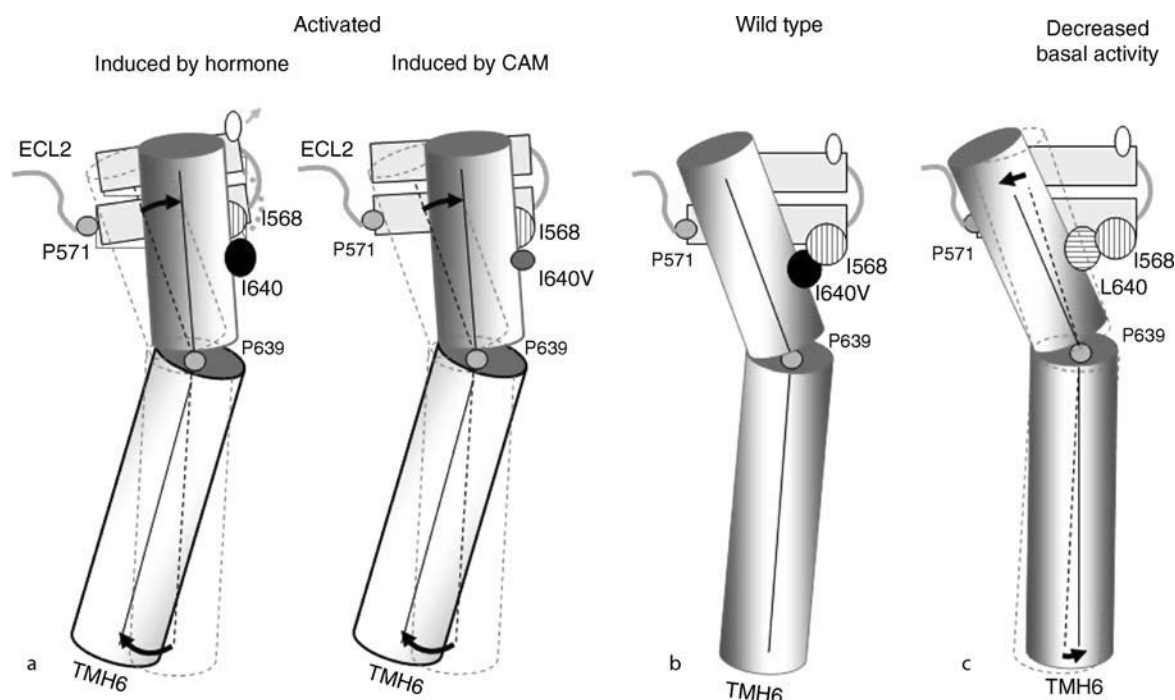
and ECL2 by introduction of mutations in TSHR can either increase or decrease basal activity and indicates its pivotal role in intramolecular signal transduction and supports the established common mechanism of an activation-associated seesaw movement of TMH6 for family A of GPCRs (Fig. 2).

Structural similarity can sometimes be a very strong indicator of similar function, but does not mean, having similar function necessarily. More important structural similarity can show evolutionary links between proteins whose ancestors may have had similar functions.

Several different types of protein domains are known to function in binding to phosphotyrosine, including



Molecular Modeling. Figure 1 Molecular model image of the environment of ECL2 in the wild type TSHR. The homology model is based on the X-ray structure of inactive bovine rhodopsin (1F88, 1HZX, 1L9H). Considering for TSHR the ECL2 partial sequence similarity, the complementarity of the environmental residues and the conserved disulfide bridge of ECL2-TMH3, a similar embedding of ECL2 into the TM bundle is feasible like in rhodopsin. Since the ECL2 sequence of TSHR is four residues shorter than the rhodopsin sequence the tip of ECL2 is slightly shortened and therefore enable an interaction between I568 (green bold) of ECL2 (red) and I640 (green bold) of TMH6 (violet), which was confirmed by mutation data. Color code of amino acids: green, hydrophobic; cyan, aromatic; yellow, cysteines; orange, hydrophilic serine and threonine; red, negatively charged; blue, positively charged.



Molecular Modeling. Figure 2 Schematic molecular mechanism for TSHR: ECL2 regulates the TMH6 adjustment and influences different activity states, which is concluded from modeling driven mutations. The generated mutants are reflecting an interface where TMH6 is gliding along ECL2 in a raster of three different receptor states. (a) in the activated state, the rather constrained basal state is released either by constitutive active mutants (CAM, E640V) with reduced side chain length/bulk or by a hormone-induced ECL2 shift (grey arrow). This is consistent to the established activation-associated see-saw movement of TMH6 (black arrows), enabling enhanced G-protein interaction for GPCRs of family A. (b) the hydrophobic side chain interaction of Ile-640 (TMH6) and Ile-568 (ECL2) is constraining the basal (partially active) conformation of wild type TSHR. (c) the decreased basal activity is provoked by a differently branched and bulkier side chain (I640L) at TMH6 by further interlocking with Ile-568, which likely causes a slight movement of TMH6 in the opposite direction to the activation conformation and diminishes G-protein interaction. For clarity only TMH6 and ECL2 of the heptahelical receptor are visualized.

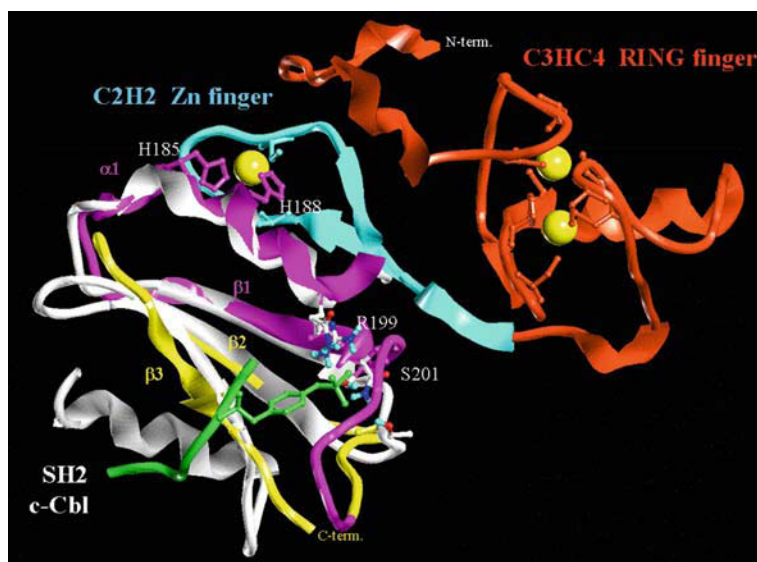
►src homology-2 (SH2) domain and ►PTB domain.

For example, the crystal structure of the amino-terminal domain from the human signaling protein Cbl revealed the presence of a cryptic SH2 domain followed by a C3HC4 ring finger domain. The sequence of this SH2 domain was so divergent that its existence had not been previously suspected. The conservation of unique and important structural elements in the X-ray structure, however, identified it clearly as a member of the SH2 family. A recently isolated E-cadherin binding protein called Hakai contained according to sequence similarity a C3H4 ring finger domain and a small portion of an incomplete SH2 domain. These are similar domains as in Cbl but with inverse order and the sequences around the incomplete SH2 domain were even more divergent from canonical SH2 domain and also from that of Cbl. Combination of sequence and fold similarity with structural alignments and functional investigation showed that Hakai is a Cbl-like protein where the SH2 domain and ring finger domain are in the same spatial arrangement. A two stranded beta-sheet identified in

the fibronectin structure by sequence similarity to Hakai replaced exactly the missing part in the incomplete SH2 fold. Although divergent to SH2 sequences the particular portion resembles exactly the corresponding SH2 fold. Moreover, an additional incomplete C2H2 Zn finger domain between ring finger and SH2 domain could be assigned to a complete Zn finger by its structural merging with the first helix of the SH2 domain, carrying the two missing histidines (Fig. 3). Mutations and functional characterization of the two histidines participating in Zn finger formation as well as the suspected arginine and serine for phosphotyrosine binding of SH2, confirmed the structural model of Hakai [3].

Simulation of Functional Properties

With growing computer power, the abilities to simulate functional properties and dynamics of fast biological reactions are increasing. Today molecular dynamics can be traced over a time range of about 1 ms. Relevant dynamics of fast biological processes (vision and



Molecular Modeling. Figure 3 Molecular model of Hakai based on structural bioinformatics, molecular modeling and biochemical experiments. Comparison of the structural model of Hakai, incomplete SH2 domain (magenta) with the SH2 domain X-ray structure of Cbl (white). The missing beta-strand fold $\beta 2$, $\beta 3$ at the incomplete SH2 sequence is exactly replaced by a beta-sheet fold found in Fibronectin (yellow) with similar sequence to Hakai in that portion. The SH2 like fold of Hakai is assembled by sequence- and fold similarity. An incomplete C2H2 Zn finger domain (blue) is completed by merging with helix $\alpha 1$ of the SH2 domain. Side chains H 185, H188 of the merged Zn finger domain and R199 and S201 of the P-Tyrosine binding site showed on mutations strong sensitive functional influences and confirm indirectly the proposed model at that region. Red, the preceding ring finger domain; green, putative phosphotyrosine peptide of E-cadherine.

photosynthesis) like electron transfer reactions, proton translocation (bacteriorhodopsin) and ion transport (potassium channels) in proteins have been studied. MD simulations can provide a realistic description of the actual reactive event.

Recently the dynamics and mechanisms of water permeation through biological membranes via pore proteins were studied using Aquaporin 1 (AQP1) and the homologous glycerol facilitator (GlpF). The selective pattern for transport of water at AQP1 and glycerol at GlpF could be identified. Also a fine-tuned water dipole rotation during the passage through the pores could be simulated by MD [4].

Critical examinations of dynamic effects showed that they are rather unlikely contribute to processes with significant activation barriers. Even in cases of ion channels it is found that the most important effects are associated with energies rather than dynamics [5].

The resulting insight of MD is crucial in studies of fast photo biological reactions and instructive in cases of slower processes. Very slow processes like folding of proteins cannot be traced by MD, because folding takes a time range between 20 ms and 1 h.

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Molecular Simulation

- G-protein-coupled receptors
- Bioinformatics
- NMR-Based Ligand Screening

- Molecular Modeling

Monoamine Oxidases

► Monoamine Oxidases and their Inhibitors

Monoamine Oxidases and their Inhibitors

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Synonyms

Monoamine oxidases; Inhibitors; Depressive illness;
Parkinson's disease; Neuroprotection neurorescue

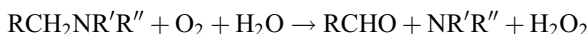
Definition

Ubiquitous mitochondrial monoamine oxidase [monoamine oxygen oxidoreductase (deaminating) (flavin-containing); EC 1.4.3.4; MAO] exists in two forms, namely type A and type B [► [monoamine oxidase \(MAO\) A and B](#)]. They are responsible for oxidative deamination of primary, secondary, and tertiary amines, including ► [neurotransmitters](#), ► [adrenaline](#), ► [noradrenaline](#), ► [dopamine \(DA\)](#), and ► [serotonin](#) and ► [vasoactive amines](#), such as tyramine and phenylethylamine. Their nonselective and selective inhibitors (► [selective MAO-A and -B inhibitors](#)) are employed for the treatment of ► [depressive illness](#) and ► [Parkinson's disease \(PD\)](#).

Reaction Mechanism of MAO

The role of MAO in terminating the action of the aminergic neurotransmitters and dietary amines has

been extensively studied and less attention has been paid to the functions of the products formed in the MAO reactions that include aldehyde and acidic metabolites.



Each of the products of this reaction may have important metabolic and signaling functions [1], but they are also potentially toxic at higher concentrations. The hydrogen peroxide resulting from MAO-catalyzed substrate oxidation has been shown to mimic the action of insulin, in adipocytes and cardiomyocytes and some other cell types, by increasing the number of glucose transporters at the cell surface, but the physiological significance of this is unknown [1]. It has been suggested that the aldehydes derived from serotonin and noradrenaline deamination may be involved in the regulation of sleep. On the other hand, aldehyde derived from dopamine, which never accumulates in normal brain, is thought to be highly toxic to dopamine neurons of substantia nigra in ► [Parkinson's disease](#), where aldehyde dehydrogenase is almost absent in parkinsonian substantia nigra pars compacta and its ► [6-hydroxydopamine](#) model. Such aldehydes may also form adducts with amine groups, to yield compounds such as *N*-methyl-R-salsolinol and tetrahydropapaveroline and peptide adducts, which may have pathophysiological roles.

Basic Characteristics

MAO is an outer mitochondrial membrane enzyme, which possess mole/mole of enzyme, covalently bound ► [FAD \(flavin adenine dinucleotide\)](#) as cofactor. Although it had been shown that MAO was not a single enzyme, its differentiation into two forms was first identified by enzyme inhibitory sensitivity in the presence of different substrates. The irreversible propargylamine inhibitor, clorgyline, inhibits serotonin and noradrenaline metabolism at low concentrations in a sigmoidal fashion, although it is a poor inhibitor of benzylamine and phenylethylamine oxidation. However, with dopamine and tyramine a double sigmoidal inhibition is observed. This has been interpreted as representing two enzymes termed A and B, where

Monoamine Oxidases and their Inhibitors. Table 1 Substrate specificity of the two forms of rat liver and brain monoamine oxidase

Substrates of the A-form	Substrates of both forms	Substrates of the B form
Adrenaline	Dopamine	Benzylamine
Noradrenaline	Tyramine	Phenylethylamine
3-O-Methyladrenaline	3-Methoxytyramine	Phenylethanolamine
3-O-Methylnoradrenaline	<i>p</i> -Synephrine	O-Tyramine
		1,4-Methylhistamine
		<i>N</i> -Tele-methylhistamine
Serotonin		

Monoamine Oxidases and their Inhibitors. Table 2 Kinetic parameters of the two forms of monoamine oxidase

Substrate	Source	Type A K_m (μ M)	Type B K_m (μ M)
Serotonin	Rat liver	160	2,000
Serotonin	Rat brain	180	1,200
Serotonin	Human platelet		1,800
Serotonin	Bovine chromaffin		2,100
Noradrenaline	Bovine chromaffin		1,750
Phenylethylamine	Human platelet		16
Phenylethylamine	Bovine chromaffin		21
Tyramine	Rat liver	110	580
Phenylethylamine	Rat liver	280	20
Dopamine	Human cerebral cortex	212	220
	Human striatum	180	210
Noradrenaline	Human cerebral cortex	284	238

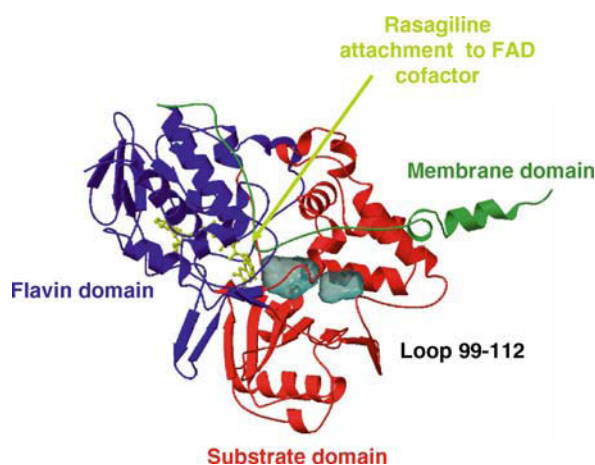
clorgyline is an inhibitor of MAO type A and its substrates are serotonin and noradrenaline (Tables 1 and 2). By contrast the irreversible propargylamine inhibitor selegiline (*L*-deprenyl) is a potent inhibitor of Type B, which metabolizes benzylamine and phenylethylamine (Tables 1 and 2). MAO-A and -B have been cloned and shown to be different proteins with 70% homology of B form with that of the A form, but with the same cysteinyl-covalently bound flavin (Fig. 1). The propargylamine derived irreversible MAO-A and -B inhibitors bind covalently to the *N*-5 isoalloxazin moiety of the flavin covalently mole/mole of the enzyme (Fig. 2) [2, 3, 4].

Drugs

The differentiation of MAO employing clorgyline and selegiline has been employed to identify the presence of each form in different tissues and species (Table 3) [4, 5]. The same system has been employed with substrates of MAO-A and -B (Table 1) to identify selective and specific inhibitors (Table 4, Fig. 2) and substrates (Table 2). The selective propargylamine inhibitors (clorgyline, selegiline, rasagiline, *N*-(2-propynyl)-2-(5-benzyloxy-indolyl) methylamine, aliphatic *N*-methyl propargylamine) lose their selectivity at higher concentration both in vitro and in vivo. By contrast the reversible inhibitors (Table 4) appear to be relatively more specific in vitro and in vivo inhibition [1].

Neuropharmacology of MAOs and their Inhibitors

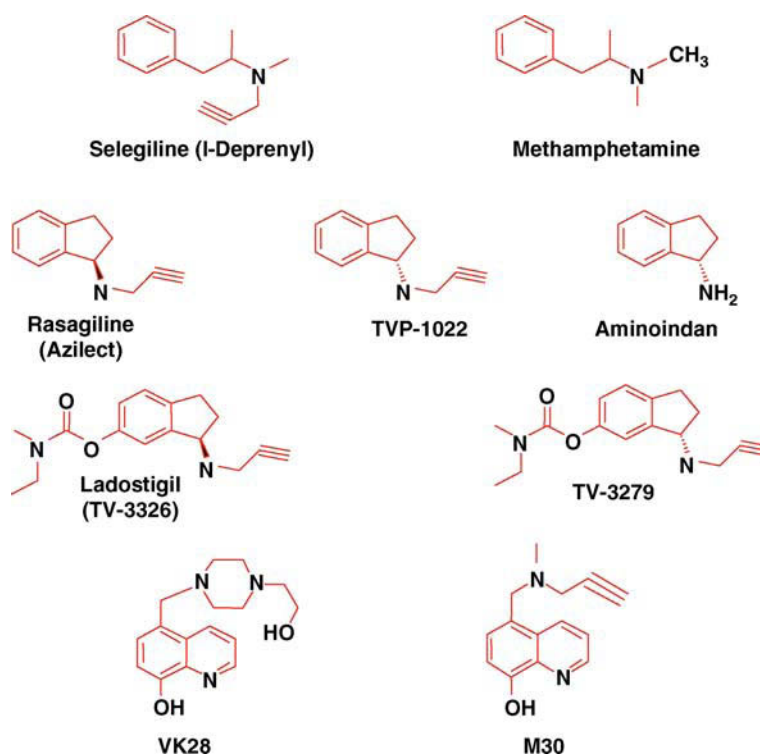
Acute treatment with nonselective MAO inhibitors (iproniazid, tranylcypromine, phenelzine), as a consequence of inhibiting both forms of the enzyme, increase brain levels of all monoamines (phenylethylamine, tryptamine, methylhistamine aminergic neurotransmitters (dopamine, noradrenaline, adrenaline and serotonin). By contrast MAO-A inhibitors (clorgyline) increase serotonin and noradrenaline, while MAO-B inhibitors (selegiline, rasagiline) increase brain levels



Monoamine Oxidases and their Inhibitors.

Figure 1 The crystal structure of human MAO-B. There are three functionally distinct domains, as shown. In red, the substrate domain contains two "cavities" shown in cyan. The outer space is the entrance cavity leading to the inner space, substrate binding cavity, closer to the flavin cofactor. The flavin-binding domain is shown in blue with the FAD molecule in yellow. In green, the C-terminal helical region which attaches the protein to the mitochondrial membrane. Rasagiline covalently links to the flavin via its propargylamine group (yellow arrow) and the indan ring extends into the substrate-binding cavity, blocking access for substrate.

of phenylethylamine and related amine. Neither class of the selective inhibitors affects brain levels of dopamine. Since the latter neurotransmitter is equally well metabolized by both enzymes. Thus when one form is inhibited, the other continues its degradation. A combination of MAO-A plus MAO-B inhibitors have the same effect as the nonselective inhibitors. Several studies in vitro and in vivo have shown that serotonin and noradrenaline are not strictly metabolized by



Monoamine Oxidases and their Inhibitors. Figure 2 Structures of MAO inhibitors. In the top row, the structural similarity between selegiline/L-deprenyl and methamphetamine is shown. Below are the aminoindan series of propargylamine compounds such as rasagiline. Next, the bifunctional MAO and cholinesterase inhibitors (ladostigil) and lastly, the iron chelator-MAO inhibitors.

Monoamine Oxidases and their Inhibitors. Table 3 Distribution and proportions of the A and B forms of monoamine oxidases in adult rat and human tissues

	% A and B form activity			
	Rat		Human	
	A	B	A	B
Kidney	70	30	57	40
Liver	55	45	54	50,45
Spleen	95		34	70
Intestine	70	30	80	25
Lung	50			
Testis	90	10		
Vas deferens	50	50		
Brain	55	45		
Primate brain				
Human basal ganglia			20	80
Monkey striatum and basal ganglia			20	80
Superior cervical ganglion	90	10		
Pineal body	15			
Heart	98, 65		43	
Aorta			48	
Adrenal gland			60	

Monoamine Oxidases and their Inhibitors. Table 3 Distribution and proportions of the A and B forms of monoamine oxidases in adult rat and human tissues (Continued)

	% A and B form activity			
	Rat		Human	
	A	B	A	B
Adrenal chromaffin cells				
Human			0	>90
Bovine			0	>98
Ileum	78	22		80
Skeletal muscle			28	
Skin			31	
Human platelet			0	>98

Monoamine Oxidases and their Inhibitors. Table 4 Nonselective and selective monoamine oxidase inhibitors

	MAO type		
	A + B	A	B
Irreversible			
Iproniazid	+AD		
Phenelzine	+AD		
Isocarboxazide	+AD		
Tranylcypromine	+AD		
	AD/APD?		
Nialamide	+AD		
Pargyline	AD	AD	>B
Clorgyline		+AD	
Deprenyl (Selegiline)			+AD/APD
Rasagiline (Azilect)			+AD/APD?
Ladostigil (TV3326)	+AD, AAD/APD? (Brain selective)		
M30	+AD, AAD/PD? (Brain selective)		
Aliphatic <i>N</i> -methyl propargylamines			+
<i>N</i> -(2-propynyl)-2-(5-benzyloxy-indolyl) methylamine			+
Reversible			
Moclobemide		+AD, APD	
Brofaromine		+AD	
Caroxazone		+	
Toloxatone		+AD	
BW 1370U87		+	
Befloxatone		+AD	
Lazabemide			+APD
Safinamide			+APD
Milacemide			+

AD, antidepressant; APD, antiParkinson; >B more B inhibitor.

MAO-A only. When concentration of these amines is high enough to satisfy the K_m of MAO-B for these amines, they can be metabolized by this enzyme form [4]. A similar picture is also seen in vivo. For this reason,

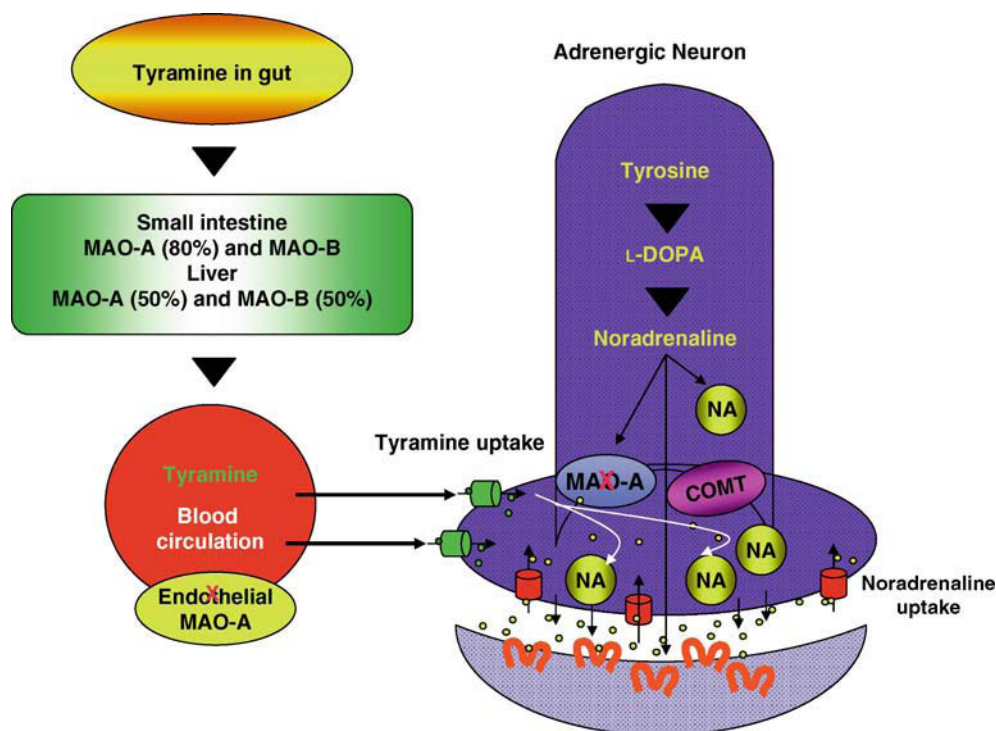
selective MAO-A inhibitors do not increase the brain levels of serotonin and noradrenaline to the same extent as nonselective inactivators [4, 5]. The acute effects of MAO inhibitors on amine neurotransmitter metabolism

are not mimicked on chronic treatment, since these amines cause a feed-back inhibition of the rate limiting synthetic enzymes, tyrosine hydroxylase (TH) and tryptophan hydroxylase. Furthermore, chronic treatment can result in increased release of noradrenaline and serotonin and inhibition of serotonin neuron firing, resulting from downregulation of presynaptic receptors alpha 2 receptors and desensitization of postsynaptic beta receptors, long term postsynaptic adaptive changes and subsensitivity of 5-HT₁ receptors. The mechanism of antidepressant actions of nonselective and MAO-A inhibitors has been linked to these effects and are seen 2–3 weeks of drug treatment, which is similar to time course of their clinical efficacy.

Cheese Reaction

Iproniazid was among the first psychotropic antidepressants to be discovered and introduced into the clinic. Although this inhibitor and others that followed

(tranylcypromine, phenelzine) demonstrated antidepressant activity, they exhibited a major side effect that came to be known as the “cheese reaction” [3, 4, 5]. The cheese reaction (Fig. 3) is induced by tyramine and other indirectly acting sympathomimetic amines present in food (most commonly in certain cheeses, hence the name) and fermented drink, such as beer and wine. Under normal circumstances, such dietary amines are extensively metabolized by MAO-A in the gut wall and in the liver and they are thus prevented from entering the systemic circulation. In the presence of an MAO inhibitor, this protective system is inactivated and tyramine or other monoamines and their amino acid precursor that can be decarboxylated, present in ingested food, enter the circulation without being metabolized. From here they have access to peripheral adrenergic neurons from which they induce a significant release of noradrenaline. The peripheral adrenergic neurons contain solely MAO-A, which is also



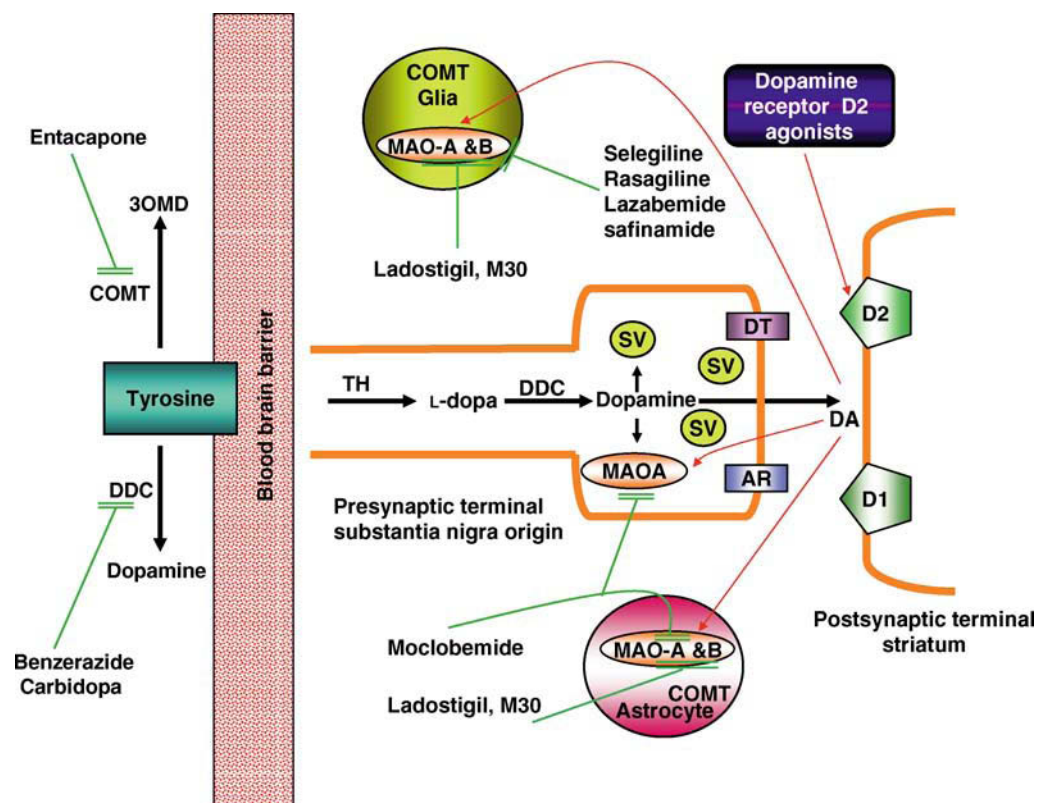
Monoamine Oxidases and their Inhibitors. Figure 3 The “cheese reaction” – potentiation of cardiovascular effects of tyramine (or other indirectly acting sympathomimetic amines) by irreversible inhibitors of MAO. Normally, dietary tyramine undergoes extensive “first pass” inactivation by the MAO-A and -B types in gut wall and then in the liver. The tyramine that survives to enter the systemic circulation is further inactivated by the MAO in vascular endothelial cells and lung (Bakhle, 1990). At the adrenergic neurone, uptake of tyramine initiates the release of noradrenaline, which accounts for the sympathomimetic effects of tyramine. Irreversible inhibition of MAO-A, the predominant form in the periphery adrenergic neurons, allows greatly increased tyramine entry in the systemic circulation and from there, adrenergic neurons, consequently increasing noradrenaline release and effect. By contrast, reversible inhibitors of MAO-A (RIMAs) are displaced from the enzyme by tyramine which is then metabolized normally by the enzyme. Thus, circulating tyramine never attains the high levels resulting from irreversible inhibition of MAO.

inhibited. The consequence of this release is a severe hypertensive response which can, in some cases, be fatal. These serious side effects stimulated a search for antidepressants that were not MAO inhibitors and led to their eventual replacement by the uptake inhibitors, the tricyclic antidepressants, and more recently the serotonin selective reuptake inhibitors (SSRI) such as Prozac. Better understanding of MAO-A and -B reactions and their distribution in peripheral adrenergic neurons had led to the demonstration that the cheese reaction is the property of irreversible MAO-A inhibition in the gut and peripheral adrenergic neurons, where this enzyme resides. The irreversible MAO-B inhibitors such as L-deprenyl (selegiline) and rasagiline (Azilect) do not induce cheese effect and have limited cardiovascular potentiation at their MAO-B inhibitory dosage. However, at inhibitor concentrations where this is lost and MAO-A is inhibited, the cheese reaction is

prominent. These studies resulted in the development of reversible MAO-A inhibitors (moclobemide) as ►**anti-depressant** that would not give a cheese reaction. The logistic being that tyramine taken up by adrenergic neurons can compete with the reversible MAO-A inhibitors at the active site of MAO, it can be metabolized before it can induce release of noradrenaline [3, 4].

MAO Inhibitors as Therapeutic Agents Parkinson's Disease

As early as 1961, the first generation of MAO inhibitors (iproniazid, isocarboxazide) were employed for the treatment of Parkinson's disease (PD). However, because of the severe side effects, such as "cheese reaction," they were abandoned. The realization that the basal ganglia (extrapyramidal region) of human brain contained mostly MAO-B, which metabolized



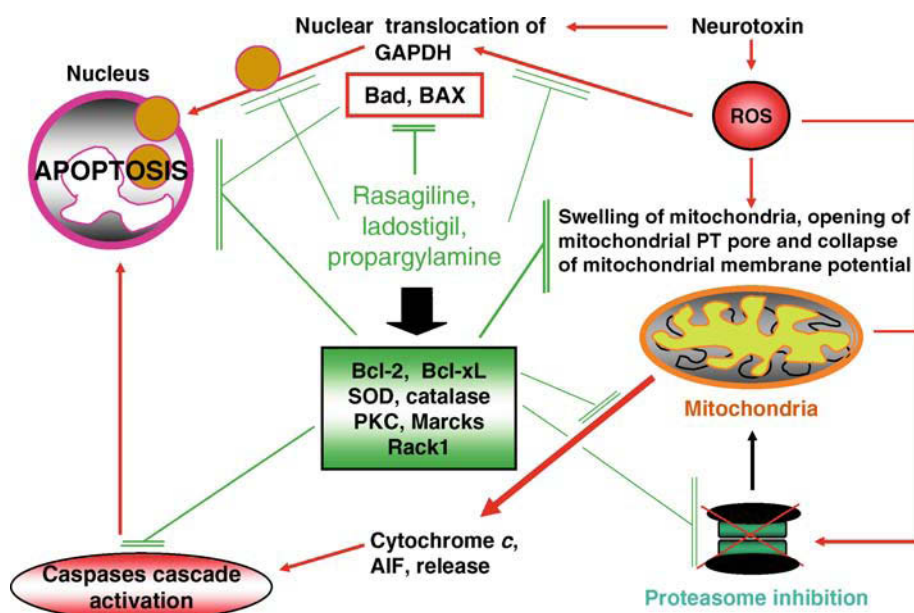
Monoamine Oxidases and their Inhibitors. Figure 4 Pathways of dopamine synthesis in dopaminergic neurons and metabolism by MAO-A and -B in the brain. Tyrosine passes through the blood–brain barrier and is hydroxylated by tyrosine hydroxylase (TH) to DOPA and then decarboxylated by DOPA decarboxylase (DDC) to dopamine (DA) within the neuron. Dopamine is taken up into synaptic vesicles (SV) or metabolized by neuronal mitochondria MAO-A. After release from the terminal, extracellular dopamine is cleared by uptake into astrocytes and glia also containing MAO-A and MAO-B. Selective inhibition of one MAO isoform allows the other to metabolize dopamine effectively and does not alter the steady state levels of striatal dopamine. On the other hand, nonselective inhibition of MAOs induces highly significant increase in striatal dopamine and in other brain regions, D1 and D2, dopamine receptors. On the other hand, in adrenergic and serotonergic neurons MAO-A and not MAO-B inhibition results in increased levels of these amines.

dopamine and that the MAO-B inhibitor, L-deprenyl (selegiline), did not potentiate the sympathomimetic actions of tyramine in isolated preparations or its cardiovascular effect in vivo, led to its employment as adjuvant to L-dopa in PD in 1975. Confirmation of these early results and its effectiveness in monotherapy led to its introduction into the clinic. Confirmation that MAO-B inhibitors' ►symptomatic activity as antiParkinson has come from the development of other such drugs (many of which were subsequently abandoned because of toxicity). However, a recent addition into the clinic is the second generation of MAO-B inhibitor, rasagiline (Azilect). The mechanism of the antiParkinson action of MAO-B inhibitors is not fully known, but may be related to increased dopamine or to the ability of elevated phenylethylamine to release dopamine. It is logical that since dopamine is metabolized both by MAO-A and -B, the MAO-A inhibitors would also have antiParkinson activity (Fig. 4). However, because of the cheese reaction, irreversible MAO-A or MAO-AB have not been employed. By contrast the few clinical studies so

far done with the reversible MAO-A inhibitor, moclobemide, have shown that it does possess antiParkinson activity, without inducing a cheese reaction [5].

Neuroprotection in Neurodegenerative Diseases

The ►neurotoxin MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) can cause parkinsonism in human and nonhuman primates and rodents by its ability to degenerate nigrostriatal dopamine neurons and deplete dopamine. MPTP is an inert tertiary amine which is metabolized by MAO-B to the neurotoxin ►MPP⁺ and MAO-B inhibitors prevent this and the neurotoxic effects of MPTP in animals and neuronal cell culture studies. This suggested that PD could be a result of an environmental neurotoxin, however, no such neurotoxin has so far been discovered, since MPTP is a synthetic substance. Nevertheless, propargyl possessing MAO-B inhibitors such as selegiline and rasagiline have received much attention as ►neuroprotective drugs, because they have neuroprotective activity unrelated to MAO-B inhibition in neuronal cell cultures and in vivo. These



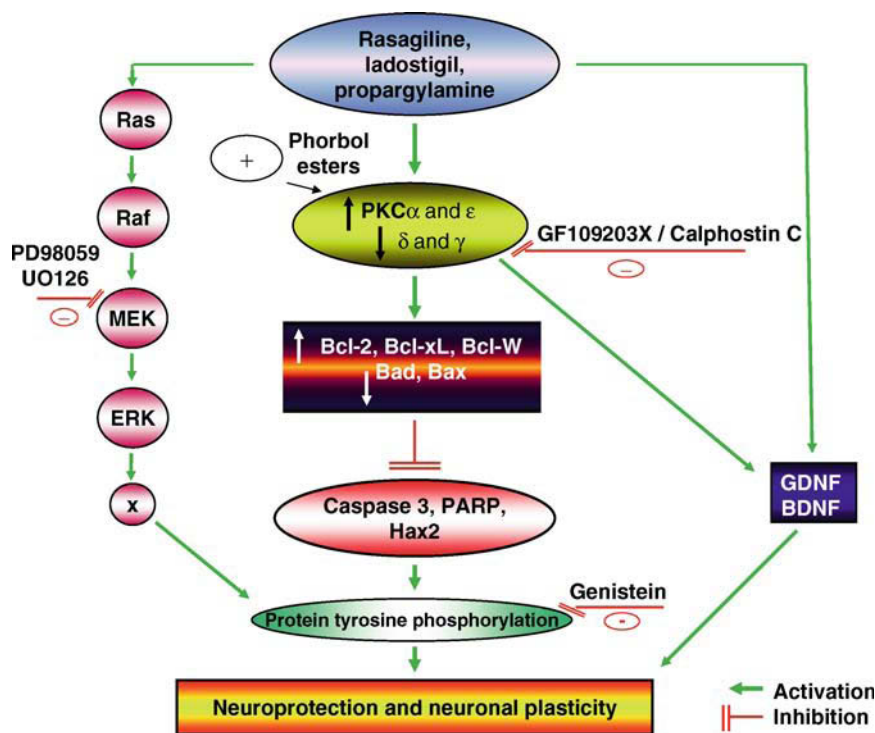
Monoamine Oxidases and their Inhibitors. Figure 5 The interactions of irreversible, propargylamine-based, MAO-B inhibitors with apoptotic pathways. Mitochondria are responsible for cell survival and death through the regulation of the B-cell leukemia/lymphoma 2 (BCL2) family antiapoptotic (BCL2) and proapoptotic (BCL-associated death promoter, BAD and BCL2-associated protein X, BAX) proteins. Rasagiline, selegiline, ladostigil, and propargylamine have been shown to induce cell survival in response to serum withdrawal or neurotoxins in neuronal cell cultures (SHSY-5Y and PC-12) through the activation of BCL2 and BCL-XL and the downregulation of BAD and BAX. These propargylamines exert their neuroprotective activity by interacting with the mitochondrial outer membrane. The propargylamine moiety in these inhibitors prevents neurotoxin-induced collapse of mitochondrial membrane potential, mitochondrial permeability transition, and the opening of the voltage-dependent anion channel, as a consequence of the upregulation of antiapoptotic BCL2 family proteins, which also lead to disinhibition of proteasome function. In addition, these propargylamines prevent the nuclear localization of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in response to neurotoxins and reactive oxygen species (ROS). AIF, apoptosis-inducing factor; PKC MARCKS, myristoylated alanine-rich protein kinase C (PKC) substrate; RACK1, receptor of activated PKC; SOD, superoxide dismutase.

drugs upregulate ►**antiapoptotic** B-cell leukemia/lymphoma 2 (BCL2), while downregulating proapoptotic BCL2 family proteins (Fig. 5) and activate PKC-MAP (protein kinase C-mitogen-activated protein) kinase pathways (Fig. 6). The result is prevention of the demise of mitochondrial and its permeability transition and cell survival. This property has been directly linked to their propargyl moiety since propargylamine, which is devoid of MAO inhibitory activity, has similar effects. The suggestion that selegiline and rasagiline may be neuroprotective in PD, with their ►**disease modifying** action, has not been shown directly but is under investigation. However, a recent delayed-start study, in which rasagiline was given to two groups of parkinsonian subjects 6 months apart, has shown that subjects treated with rasagiline for 12 months showed less

functional decline than subjects whose treatment was delayed for 6 months. This has been suggested to indicate a disease modifying property. A major controlled study is under way to confirm this [4, 5].

Depressive Illness

Although it could be fatal, the cheese reaction seen with the first generation of MAO inhibitors as antidepressant was rather exaggerated. Not all patients that consumed food stuff containing sympathomimetic agents reacted in this manner. Nevertheless the irreversible MAO-AB inhibitors tranylcypromine and phenelzine are still in clinics, with caution taken that patients avoid certain food stuff containing tyramine. With the reversible MAO-A inhibitor, moclobemide (Fig. 4), this is unnecessary since it has highly limited capacity to induce the cheese reaction.



Monoamine Oxidases and their Inhibitors. Figure 6 The mechanism of neuroprotection and/or neurorescue by propargylamine MAO inhibitors. The propargylamine monoamine oxidase (MAO) inhibitors (such as rasagiline and ladostigil) activate the protein kinase C (PKC)-dependent mitogen-activated protein (MAP) kinase pathway 185,187 and mitochondrial B cell leukemia/lymphoma 2 (BCL2) family cytoprotective proteins, and down regulate proapoptotic BCL-associated death promoter (BAD), BCL2-associated protein X (BAX), caspase 3, poly (ADP-ribose) polymerase 1 (PARP1) and H2AX (a phosphorylated mammalian histone H2A)141. One direct consequence is the processing of amyloid precursor protein (APP) through the activation of α -secretase, resulting in increased release of the neuroprotective/neurotrophic soluble APP α (sAPP α). The activation of PKC α and PKC ϵ might explain the ability of these propargylamines to induce expression of glia cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) mRNAs, and stimulate the release of GDNF. Inhibitors of PKC (such as GF109203X and calphostin C), extracellular signal-regulated kinase 1/2 (ERK1/2) (e.g., PD98059 and UO126) and tyrosine kinase (for instance, genistein) prevent APP processing and release of GDNF, and block BCL2-dependent neuroprotective pathways, supporting a mechanism by which propargylamines operate at different levels and suggesting a direct link between PKC and BCL2 family protein expression. MEK, MAP kinase; Raf, Ras, small G proteins; X represents a phosphorylated protein that activates tyrosine kinase.

Because of its mild antidepressant activity, it is generally considered to be more suitable for elder subjects, who react poorly to standard antidepressants [3, 4].

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Monoclonal Antibody

Monoclonal antibodies are derived from a single, monospecific B cell clone. Monoclonal antibodies can be obtained from hybridoma cells that result from the fusion of antibody-producing B cells with immortal cells of a myeloma cell line.

- T Cell Receptors
- Humanized Monoclonal Antibodies

Monocytes

- Inflammation

Monokines

- Cytokines

Monophasic Preparations

In monophasic preparations a fixed estrogen/progestin combination is present in each contraceptive pill.

- Contraceptives

Monoxide

- Carbon Monoxide

Mood Disorders

- Affective Disorders

Mood Elevators

- Antidepressant Drugs

Mood-stabilising Drugs

In contrast to antidepressants (mood-elevating agents), mood-stabilizing drugs are not only effective in treating a state of depression, but can also control the manic phase of a manic-depressive (bipolar) illness. Mood-stabilizing drugs are often used prophylactically in bipolar depression. The most important mood-stabilizing agents are lithium salts which are able to prevent swings of mood in bipolar depression. In the acute phase, lithium is effective only in reducing mania, but not depression. It is currently not clear how lithium exerts its mood-stabilizing effects. Two main molecular mechanisms have been preposed. Lithium has been shown to inhibit inositol phosphatases. This results in a reduced

formation of free inositol, which is required for the formation of phosphatidyl inositols, serving as substrates for phospholipases (e.g. phospholipase- β). Lithium, thus, blocks the phosphatidyl inositol (PI) pathway resulting in the inhibition of agonist-stimulated inositol 1,4,5-triphosphate (IP₃) formation by a variety of receptors. Recently lithium has been shown to inhibit glycogen synthesis kinase-3 β (GSK-3 β), which is involved in various neuronal regulatory processes, including the Wnt-pathway.

► Antidepressants

Morbus Alzheimer

► Alzheimer's Disease

Morphogens

Secreting signaling molecules with a pivotal role in organ development, particularly directing the positions of the specialized cell types in an organ. Examples of morphogens include transforming growth factor- β , fibroblast growth factor, and Wnt.

► Transforming Growth Factor-Beta

Morpholines

Morpholines are a chemical class of organic compounds with the six-membered ring tetrahydro-1,4-oxazine, as their basic structure.

► Antifungal Drugs

Motilin

Motilin is a 22-amino acid peptide hormone, secreted by the enterochromaffin cells of the small intestine,

which exerts a profound effect on gastric motility by inducing contractions of the antrum and duodenum. Motilin acts on a G-protein coupled receptor, which is expressed in a subset of interstitial cells of the human duodenum, jejunum and colon. Macrolide antibiotics like erythromycin and roxithromycin act as agonists on the motilin receptor.

► Macrolides

Motion Sickness

► Emesis

Motor Proteins

Motor proteins move along microtubules or F-actin. The respective motor domains are linked to their cargoes via adaptor proteins. Kinesin motors move only to the plus and dynein motors only to the minus ends of microtubules. Myosin motors move along F-actin. When motors are immobilized at their cargo binding area, they can move microtubules or F-actin, respectively.

► Cytokeratin ► Cytoskeleton

MPEP

► 2-Methyl-6-(Phenylethynyl) Pyridine

MPP⁺

► 1-Methyl-4-Phenylpyridinium

MPTP

Synonyms

N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

Definition

A synthetic neurotoxin that causes parkinsonism in human and nonhuman primates, mice, gold fish, and dogs. MPTP is inert but metabolized by MAO-B to the neurotoxin MPP⁺ (1,2-dihydropyridine ion). This neurotoxin causes depletion of dopamine and degeneration of nigrostriatal dopamine neurons similar to what is observed in Parkinson's disease.

- ▶ Monoamine Oxidases and their Inhibitors
- ▶ 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
- ▶ Organic Cation Transporters
- ▶ G-Hydroxydopamine

mRNA

Messenger RNA (mRNA) is the intermediate template between DNA and proteins. The information from a particular gene is transferred from a strand of DNA by the construction of a complementary strand of RNA through a process known as transcription. The amount of any particular type of mRNA in a cell reflects the extent to which a gene has been 'expressed'.

- ▶ Microarray Technology

MRP

Multidrug resistance related protein (MRP). Currently nine MRP proteins have been described in human. The MRP proteins belong to the C-branch of the ABC-transporter superfamily and are also involved in resistance against several anticancer drugs. MRP proteins transport a wide variety of endo- and exogenous compounds. For example, MRP2, which is expressed in the canalicular membrane of hepatocytes, eliminates bilirubin-glucuronide into the bile, and MRP4, 5, and 8 have been shown to eliminate cyclic nucleotides.

- ▶ ABC-Transporter

MS/MS

- ▶ Tandem Mass Spectrometry

mTOR, replaces FRAP, RAFT, RAPT or SEP in mammals

- ▶ TOR Signalling

Mucolipin

Mucolipin, also known as mucolipin 1 or mucolipidin (encoded by the MCOLN1 gene), is a TRP channel-related membrane protein, most probably residing in intracellular membranes. Is defective in mucopolipidosis type IV disease, a developmental neurodegenerative disorder characterized by lysosomal storage disorder and abnormal endocytosis of lipids. The function of mucolipin is unknown.

- ▶ TRP Channels

Multidrug Resistance

The multidrug resistance (MDR) phenomenon has first been described in cancer cells which were resistant against several structurally unrelated anticancer drugs. Mechanistically a high expression of different ABC-transporters (P-gp, MRP) has been identified as the underlying factor.

- ▶ ABC-Transporter
- ▶ MDR-ABC Transporters

Multidrug Resistance Gene

- ▶ MDR-ABC Transporter

Multiple Sclerosis

Multiple sclerosis is an autoimmune disease mediated by T and B lymphocytes and macrophages. This is characterized by extensive inflammation and demyelination of the myelin sheath that surrounds the nerve fiber. The death of the nerve fiber results in a variety of symptoms that can lead to impairment of movement, paralysis, and death.

► Chemokine Receptors

Muscarinic Acetylcholine Receptors

► Muscarinic Receptors

Muscarinic Receptors

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Synonyms

Muscarinic acetylcholine receptors

Definition

Muscarinic acetylcholine receptors (mAChRs) form a class of cell surface receptors that are activated upon binding of the neurotransmitter, acetylcholine. Structurally and functionally, mAChRs are prototypical members of the superfamily of G protein-coupled receptors. Following acetylcholine binding, the activated mAChRs interact with distinct classes of heterotrimeric G proteins resulting in the activation or inhibition of distinct downstream signaling cascades.

Basic Characteristics

The neurotransmitter acetylcholine (ACh) exerts its diverse pharmacological actions via binding to and subsequent activation of two general classes of cell surface receptors, the nicotinic and the mAChRs. These two classes of ACh receptors have distinct structural and functional properties. The ►nicotinic receptors,

which can be selectively activated by the alkaloid nicotine, represent ACh-gated ion channels. In contrast, the mAChRs, which can be selectively activated by the alkaloid muscarine, are members of the ►G protein-coupled receptor (GPCR) superfamily.

General Structural Features

Molecular cloning studies have revealed the existence of five molecularly distinct mammalian mAChRs (M₁-M₅; Table 1; Fig. 1) [1, 2]. All five receptor subtypes are members of the so-called class I GPCR subfamily (rhodopsin-like receptors) with which they share about 20 highly conserved amino acids (Fig. 1). These highly conserved residues play important roles in proper receptor folding and receptor activation [1, 3]. The structural hallmark of mAChRs (and GPCRs in general) is the presence of seven α -helically arranged transmembrane domains (TM I-VII; Fig. 1), which form a tightly packed transmembrane core. The N-terminal portion of the receptor protein is located extracellularly, whereas the C-terminal segment protrudes into the cytoplasm. The seven transmembrane helices are linked by three intracellular (i1-i3; Fig. 1) and three extracellular loops (o2-o4; Fig. 1). A characteristic structural feature of the mAChRs is the presence of a rather large third intracellular loop (i3 loop; 157–240 amino acids in length), which, except for the N- and C-terminal segments, displays virtually no sequence homology among the different subtypes. The N- and C-terminal portions of the i3 loop play important roles in receptor/G protein coupling [1], whereas the central portions of the i3 loop are involved in the regulation of receptor activity (see below). The five receptor subtypes share the highest degree of sequence homology within the seven membrane-spanning domains (Fig. 1), which are known to be involved in ACh binding [1, 3].

Distribution and Physiological Functions of mAChRs

mAChRs are found in virtually all organs, tissues, and cell types. All five mAChRs are expressed in both the central nervous system (CNS) and the body periphery. Whereas the M₂ and M₃ receptor subtypes are the predominant mAChRs found in peripheral tissues, the M₁ and M₄ mAChRs are abundantly expressed in the CNS, specifically in higher brain regions. The M₅ mAChR is expressed at rather low levels in various central and peripheral tissues. Characteristically, most tissues or organs express multiple mAChR subtypes.

Peripheral mAChRs are known to mediate the well-documented actions of ACh at ►parasympathetically innervated effector tissues (organs) including heart, endocrine and exocrine glands, and smooth muscle tissues [2, 4]. The most prominent peripheral actions mediated by activation of these receptors are reduced heart rate and cardiac contractility, contraction of

Muscarinic Receptors. Table 1 Summary of key features of the five human mAChRs (M₁–M₅)

Receptor subtype	M ₁	M ₂	M ₃	M ₄	M ₅
Amino acids	460	466	590	479	532
Chromosomal localization	11q12	7q35–36	1q43–44	11p12–11.2	15q26
GenBank/EMBL Accession number	X15263	X15264 M16404	X15266	X15265 M16405	M80333
G protein-coupling selectivity	G _{q/11}	G _{i/o}	G _{q/11}	G _{i/o}	G _{q/11}
Subtype-preferring antagonists (examples) ^a	Pirenzepine	Tripitramine	Darifenacin	PD102807	— ^c
	MT7 ^b		MT3 ^b		

^aExcept for certain snake toxins, such as MT3 or MT7, the degree of subtype preference of so-called “subtype-selective” muscarinic antagonists is usually relatively small (for a summary of affinity values, see [2].)

^bMT3 and MT7 are toxins isolated from the venom of the green mamba (*Dendroaspis augusticeps*).

^cM₅ mAChRs are pharmacologically distinct in that they display very low affinity for antagonists such as AF-DX 116 and its derivatives [2].

smooth muscle tissues (e.g. smooth muscles of the eye, gastrointestinal system, lung, or urinary bladder), and stimulation of glandular secretion (e.g. lachrymal, salivary, and gastrointestinal glands). Whereas the cardiac muscarinic actions of ACh are mediated by M₂ receptors, the M₃ receptor subtype plays a major role in mediating ACh-dependent stimulation of glandular secretion and smooth muscle contraction [2, 4].

Central mAChRs are involved in modulating a very large number of behavioral, autonomic, sensory, and motor functions. For example, central muscarinic mechanisms play important roles in the control of body temperature, cardiovascular and pulmonary functions, learning and memory, emotional responses, arousal, attention, rapid eye movement (REM) sleep, and stress modulation. Moreover, increased or decreased muscarinic cholinergic neurotransmission has been implicated in the pathophysiology of several important disorders of the brain, including ►**Alzheimer’s disease** and ►**Parkinson’s disease**, depression, schizophrenia, and epilepsy. The roles of the individual mAChRs in mediating the diverse central muscarinic functions of ACh are not well understood at present, primarily due to the lack of muscarinic ligands endowed with a high degree of receptor subtype selectivity (see below). However, recent studies analyzing newly developed mutant mouse strains deficient in specific mAChR subtypes have led to many new insights into the physiological roles of the M₁–M₅ receptors [4].

Ligands and Mechanisms Involved in Ligand Binding

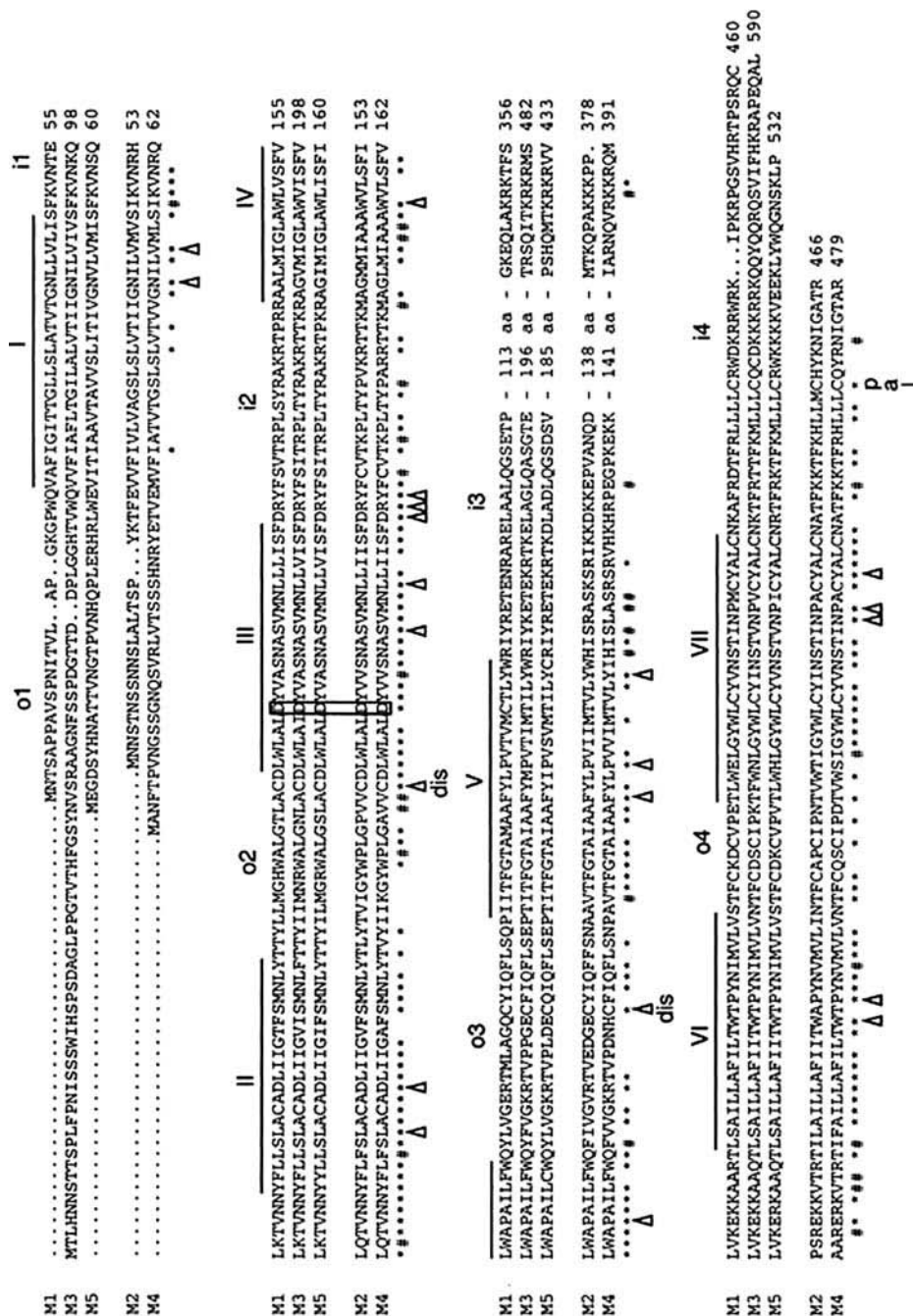
Various lines of evidence indicate that ACh binds to the M₁–M₅ receptors within a cleft enclosed by the ring-like arrangement of TM I–VII, about 10–15 Å away from the membrane surface [1, 3]. ACh binding induces as yet poorly understood changes in the arrangement of individual transmembrane helices. These conformational changes are then transmitted to the intracellular

surface of the receptor protein, enabling the receptor to productively interact with specific classes of ►**heterotrimeric G proteins**. The amino acids involved in ACh binding are located on different TM helices, primarily TM III, V, VI, and VII [1, 3]. Importantly, the positively charged ammonium head group of ACh (or the amino/ammonium head group of other classical muscarinic agonists or antagonists) is engaged in an ion–ion interaction with a TM III aspartate residue (shown boxed in Fig. 1), which is conserved among all ►**biogenic amine GPCRs**. This ion pair is surrounded by a cluster of aromatic amino acids, thus creating a charge-stabilized aromatic cage [1, 3].

The ACh-binding pocket partially overlaps with that of competitive muscarinic antagonists such as atropine, scopolamine, or quinuclidinyl benzilate. However, antagonists usually form additional strong interactions with hydrophobic receptor residues, thus stabilizing the inactive state of the receptor [1, 3].

The amino acids lining the ligand-binding cavity are highly conserved among the M₁–M₅ mAChRs. For this reason, the development of agonist or antagonist ligands able to interact with individual mAChR subtypes with a high degree of selectivity has proven to be a very difficult task. At present, agonists that display a high degree of selectivity for a particular mAChR subtype are not available [2]. Moreover, the degree of receptor subtype selectivity of so-called “selective” muscarinic antagonists, which are currently used to distinguish pharmacologically between different mAChR subtypes, is generally rather modest [2]. Such compounds include, for example, pirenzepine (M₁ receptor-preferring), tripitramine (M₂ receptor-preferring), darifenacin (M₃ receptor-preferring), or PD 102807 (M₄ receptor-preferring) [2]. Antagonists that preferentially bind to M₅ receptors are not available at present.

However, several snake toxins have been identified that display an unprecedented degree of mAChR



Muscarinic Receptors. Figure 1 Alignment of the amino acid sequences of the human M₁-M₅ mAChRs. The predicted positions of the seven transmembrane helices (I-VII) and the four intracellular (i1-i4) domains are indicated above the sequences. The central portions of the i3 loop sequences, which show very little homology among the five receptors, have been omitted. The o1 regions contain two or more consensus sites (N-X-S/T) for N-linked glycosylation (not shown). Arrowheads indicate amino acids that are highly conserved among class I GPCRs (rhodopsin family). *, amino acids identical among all five receptor subtypes. #, amino acids identical in the M₁, M₃, and M₅ mAChRs, which are replaced with different (identical) residues in the M₂ and M₄ mAChRs. The boxed TM III aspartate residue plays a key role in the binding of muscarinic ligands (see text for details). *pal*, predicted site of receptor palmitoylation. *dis*, Cys residues predicted to link the 'top' of TM III and the second extracellular loop (o3 region) via formation of a disulfide bridge.

subtype selectivity. For example, the MT7 and MT3 toxins are highly selective antagonists for M_1 and M_4 mAChRs, respectively [2]. The binding of these polypeptide ligands appears to involve interactions with less well conserved amino acids present on the extracellular surface of the mAChRs.

The binding of muscarinic ligands to the primary recognition site can be modulated by so-called allosteric ligands which interact with a secondary (allosteric) site [2]. The best known ligands of this class are certain neuromuscular blocking agents including gallamine. Most allosteric ligands exhibit negative cooperativity with classical muscarinic agonists and antagonists. However, allosteric agents that display positive cooperativity with ACh or certain muscarinic antagonists at specific mAChR subtypes have also been identified [2]. The receptor-binding site for allosteric muscarinic ligands is thought to be located just “above” the classical ligand-binding pocket.

G Protein-Coupling Properties of mAChRs

Based on their G protein-coupling properties, the M_1 - M_5 mAChRs can be subdivided into two major functional subclasses [1, 2]. The M_1 , M_3 , and M_5 mAChRs are preferentially coupled to G proteins of the $G_{q/11}$ family, which mediate the activation of different isoforms of ►phospholipase $C\beta$ resulting in the breakdown of phosphatidyl inositol and the generation of the second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. In contrast, the M_2 and M_4 mAChRs are selectively linked to G proteins of the $G_{i/o}$ class, which, at a biochemical level, reduce the accumulation of intracellular cAMP via inhibition of ►adenylyl cyclase. However, the G protein-coupling selectivity of the individual mAChRs is relative rather than absolute, as has been observed with most other GPCRs. Mutagenesis studies have shown that amino acids located within the i2 loop and the membrane-proximal portions of the i3 loop play key roles in determining the G protein-coupling profile of the individual mAChRs [1].

Signaling Pathways Activated by mAChR Subtypes

At a cellular level, the activation of mAChRs leads to a wide spectrum of biochemical and electrophysiological responses [1, 5]. The precise pattern of responses that can be observed does not only depend on the nature of the activated G proteins (receptor subtypes) but also on which specific components of different signaling cascades (e.g. effector enzymes or ion channels) are actually expressed in the studied cell type or tissue. The observed effects can be caused by direct interactions of the activated G protein(s) with effector enzymes or ion channels or may be mediated by second messengers (Ca^{2+} , IP_3 , etc.) generated upon mAChR stimulation.

Activation of M_1 , M_3 , and M_5 mAChRs does not only lead to the generation of IP_3 followed by the mobilization of intracellular Ca^{2+} , but also results in the stimulation of phospholipase A2, phospholipase D, and various tyrosine kinases. Similarly, M_2 and M_4 receptor activation does not only mediate the inhibition of adenylyl cyclase, but also induces other biochemical responses including augmentation of phospholipase A2 activity. Moreover, the stimulation of different mAChR subtypes is also linked to the activation of different classes of mitogen-activated protein kinases (MAP kinases), resulting in specific effects on gene expression and cell growth or differentiation.

Stimulation of mAChRs also results in the activation or inhibition of a large number of ion channels [5]. For example, stimulation of M_1 receptors leads to the suppression of the so-called M current, a voltage-dependent K^+ current found in various neuronal tissues. M_2 receptors, on the other hand, mediate the opening of cardiac $I_{K(ACh)}$ channels, and both M_2 and M_4 receptors are linked to the inhibition of voltage-sensitive calcium channels [5].

Regulation of mAChR Activity

Like most other GPCRs, mAChRs are subject to desensitization, which is defined as diminished responsiveness of the receptor/effector signaling pathway upon prolonged exposure of the receptor to an activating ligand. The phenomenon of GPCR desensitization involves a complex series of events, including G protein uncoupling, receptor sequestration/internalization (removal of receptors from the cell surface), and receptor downregulation associated with the net loss of receptor protein [1]. Many of these processes are regulated by receptor phosphorylation catalyzed by various protein kinases including different members of the family of ►GPCR kinases (GRKs), casein kinase 1 α , or second messenger-dependent protein kinases. Phosphorylation occurs on threonine and serine residues located within the i3 loop and the C-terminal tail of the mAChRs. The individual mAChR subtypes differ in their ability to serve as substrates for phosphorylation by these various kinases. The rapid removal of mAChRs from the cell surface following agonist stimulation (referred to as receptor internalization/sequestration) occurs through multiple pathways, one of which involves the targeting of receptors to clathrin-coated pits.

Drugs

Current Clinical Uses of Muscarinic Drugs

Muscarinic agonists and antagonists are used for the treatment of a variety of pathophysiological conditions. For example, muscarinic agonists (pilocarpine, carbachol, or aceclidine) reduce intraocular pressure when

applied locally to the eye and are therefore widely used for the treatment of glaucoma. Moreover, muscarinic agonists (carbachol or bethanechol) are employed in certain cases of atonia of the stomach, bowel, or urinary bladder. The agonists pilocarpine and cevimeline are used to stimulate salivation under conditions where the function of the salivary glands is impaired. The antagonist, scopolamine, is highly effective in preventing motion sickness. Centrally acting muscarinic antagonists (e.g. trihexyphenidyl, procyclidine, or biperiden) are useful for the treatment of Parkinson's disease or Parkinson-like symptoms caused by the administration of antipsychotic drugs, probably due to their ability to reduce excessive striatal muscarinic neurotransmission resulting from the lack of striatal dopamine. Muscarinic antagonists are also of considerable value in the treatment of clinical disorders characterized by an increased tone or motility of the gastrointestinal and urogenital tract and in the local therapy of obstructive pulmonary diseases including chronic bronchitis and bronchial asthma. Antimuscarinic agents are widely used in ophthalmology to produce mydriasis and/or cycloplegia, are effective in the treatment of peptic ulcer disease (e.g. pirenzepine) and certain forms of cardiac arrhythmias, and can be used as part of routine preoperative medication, primarily to reduce reflex bradycardia and excessive bronchial secretion.

Potential Clinical Uses of Muscarinic Drugs

A major problem associated with the use of classical muscarinic drugs is the rather common occurrence of bothersome side effects, primarily due to the stimulation or blockage of cardiac, glandular, smooth muscle, or central mAChRs. It is likely that the development of muscarinic agonists and antagonists that can interact with individual mAChRs with a high degree of selectivity will lead to novel muscarinic drugs with reduced side effects and increased efficacy. For example, it has been proposed that selective activation of central M_1 receptors or selective blockage of presynaptic M_2 receptors mediating autoinhibition of ACh release may represent potentially useful strategies for the treatment of Alzheimer's disease. Such agents could offer therapeutic benefits by facilitating signaling through cortical and hippocampal M_1 mAChRs, which lack proper cholinergic innervation in patients with Alzheimer's disease. Selective M_3 receptor antagonists are likely to produce fewer side effects in the treatment of smooth muscle disorders including urinary urge incontinence, irritable bowel syndrome, and chronic obstructive pulmonary disease. The application of subtype-selective muscarinic drugs may also be beneficial in the management of pain (centrally active muscarinic agonists are potent analgesics) and in the treatment of schizophrenia [4].

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Muscle Relaxants

Muscle relaxants reduce the tone of the voluntary muscles. Centrally acting muscle relaxants like benzodiazepines or baclofen reduce the background tone of the muscle without seriously affecting its ability to contract transiently under voluntary control. Baclofen is a selective agonist of presynaptic γ -aminobutyric acid_B (GABA_B)-receptors. Its antispastic action is due to the inhibition of the activation of motor neurons in the spinal cord. Peripherally acting muscle relaxants block neuro-muscular transmission. They either inhibit the synthesis of acetylcholine (e.g. hemicholinium) or inhibit acetylcholine release (e.g. botulinum toxin) or act postsynaptically as antagonists of the muscular nicotinic acetylcholine receptor (non-depolarising blocking agents; e.g. tubocurarine, pancuronium, vecuronium, atracurium, gallamine) or as agonists of the receptor (depolarizing blocking agents; e.g. suxamethonium). The peripherally acting relaxants are also called “neuromuscular blocking agents”.

Muscle Type Nicotinic Receptors

Nicotinic receptors (nicotinic acetylcholine receptors, nAChR) exist not only in the membrane of vertebrate skeletal muscle at the synapse between nerve and muscle (muscle-type nAChR) but also at various synapses throughout the brain, mainly at presynaptic positions (neuronal-type nAChR). Whereas the muscle-type nAChR is precisely composed of two α 1-subunits, one β -subunit, one γ -subunit and one δ -subunit (adult)

or one ϵ -subunit (embryonic), the neuronal receptors exist as homopentamers (e.g. $[\alpha 7]_5$) or heteropentamers comprising various combinations of $\alpha 2$ - to $\alpha 6$ - with $\beta 2$ - to $\beta 4$ -subunits or $\alpha 9$ - with $\alpha 10$ -subunits.

► **Nicotinic Receptors**

Myasthenia Gravis

Myasthenia gravis is an autoimmune disorder caused by antibodies to nicotinic receptors of the skeletal muscle endplate. The antibodies block the receptors directly, for example by occupying the acetylcholine binding site. They also increase the rate of degradation of the receptors. Symptoms are muscle weakness and fatigue. Cholinesterase inhibitors such as physostigmine improve muscle strength by preservation of released acetylcholine, so that a greater number of still intact receptors is exposed to effective concentrations of the transmitter.

- **Nicotinic Receptors**
- **Muscle Relaxants**
- **Synaptic Transmission**

Myelin Basic Protein

Component of the myelin sheath surrounding the axons of nerve cells. Additional compounds of the myelin sheath are phospholipids, cholesterol, cerebroside, and specific keratins. The myelin sheath constitutes an isolating barrier during electrophysiological axonal signaling.

► **Interferons**

Myelosuppression

Myelosuppression is suppression of the production of blood cells by the bone marrow.

► **Hematopoietic Growth Factors**

Myocardial Infarction

An episode of acute cardiac ischemia that leads to death of cardiomyocytes. It is usually caused by a thrombotic atherosclerotic plaque.

► **Atherosclerosis**

Myosin Light Chain Kinase

Synonyms

MLCK

Definition

This kinase specifically phosphorylates the regulatory light chain of myosin after activation by calcium-calmodulin. Several isozymes of approximately 135 kDa exist.

► **Smooth Muscle Tone Regulation**

Myosin Phosphatase (MLCP)

Smooth muscle myosin phosphatase contains three subunits, a 110–130 kDa myosin phosphatase targeting and regulatory subunit (MYPT1), a 37 kDa catalytic subunit (PP-1C) and a 20 kDa subunit of unknown function.

► **Smooth Muscle Tone Regulation**

Myotonia

Myotonia is muscle stiffness, in which muscle relaxation after voluntary contraction is impaired. Mutations in several ion channel genes (Cl, Na, Ca, K channels) can cause myotonias, which can sometimes be differentiated clinically (e.g. paramyotonia is cold-sensitive). CLC-1 mutations cause 'pure' myotonia congenita which is not sensitive to temperature. Channel myotonia comes in a recessive (Becker type)

form and a dominant (Thomsen type) form. In myotonic dystrophy (due to mutations in a kinase gene), myotonia is one of the symptoms. In contrast to myotonic dystrophy, myotonia congenita is a nondystrophic, rather benign disorder in which the skeletal muscle is not dystrophic, but rather shows hypertrophy secondary to “exercise” by prolonged contractions.

- ▶ Cl^- Channels
- ▶ Voltage-dependent Na^+ Channels

Myristoylation

Myristoylation is the post-translational addition of the 14-carbon fatty acid myristate to the N-terminal glycine of proteins via an amide link. Myristoylation of proteins helps to anchor them to membranes.

- ▶ Lipid Modifications

N-Myristoylation

► Lipid Modifications

Na⁺/Ca²⁺ Antiporter

► Na⁺/Ca²⁺ Exchangers

Na⁺/Ca²⁺ Exchanger

► Na⁺/Ca²⁺ Exchangers

Na⁺/Ca²⁺ Exchangers

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Synonyms

Sodium calcium exchanger; Na⁺/Ca²⁺ exchanger; Na⁺/Ca²⁺ antiporter; NCX; NCX1; NCX2; NCX3

Definition

The plasma membrane Na⁺/Ca²⁺ exchanger is a high-capacity and low affinity ionic transporter that exchanges three Na⁺ ions for one Ca²⁺ ion. When intracellular Ca²⁺ concentrations [Ca²⁺]_i rise and the

cells require the return to resting levels, this exchanger transport mechanism, couples the uphill extrusion of Ca²⁺ ions to the influx of Na⁺ ions into the cells down their electrochemical gradient. This mode of operation, defined as forward mode or Ca²⁺ efflux (corresponds to forward mode of operation) mechanism keeps the 10⁴-fold difference in [Ca²⁺]_i across the cell membrane. In other physiological or pathophysiological conditions when the intracellular Na⁺ concentrations [Na⁺]_i rise or membrane depolarization occurs, thus reducing the transmembrane Na⁺ electrochemical gradient, the Na⁺/Ca²⁺ exchanger mediates the extrusion of Na⁺ and the influx of Ca²⁺ ions. This mode of operation is defined as reverse mode or Ca²⁺ influx (corresponds to reverse mode of operation) mechanism. The mode of operation of the antiporter depends on: (i) the Na⁺ gradient, (ii) the Ca²⁺ gradient, and (iii) the membrane potential and therefore it is a major player in the regulation of physiological responses to increases of [Ca²⁺]_i and [Na⁺]_i [1–3].

Basic Characteristics

Molecular Biology and Topology

The molecular biology of NCX revealed that three genes coding for the three different NCX1, NCX2, and NCX3 proteins are present in mammals. These three genes appear to be dispersed, since *ncx1*, *ncx2*, and *ncx3* are localized in mouse chromosomes 17, 7, and 12, respectively. At the posttranscriptional level, at least 15 NCX1 and 5 NCX3 proteins are generated through an alternative splicing of the primary nuclear transcripts.

NCX1 protein is composed of ~938 amino acids having a theoretical molecular mass of 120 kDa and containing nine transmembrane segments named TMS 1–9. The amino terminus NCX is located in the extracellular space, whereas the carboxyl terminus is located intracellularly. The nine transmembrane segments can be divided into an N-terminal hydrophobic domain, composed of the first five: TMS 1–5, and into a C-terminal hydrophobic domain, composed of the last four: TMS 6–9. The NCX protein amino acid sequence found between TMS2 and TMS3 is called α-1 repeat, whereas the one found between TMS7 and TMS8 is named α-2 repeat. Both regions, α-1 and α-2 repeats, are located on the opposite site of the membrane. Since the

putative α -helices of the α -repeats are amphipathic, the hydrophilic faces of these helices may form a portion of the ion translocation pathway. Interestingly, the α -repeats form reentrant loops of NCX1 that interact within the protein. Interestingly, the center of α -2 repeat possesses the GIG aminoacid sequence similar to the GYG sequence present in the P loop of K^+ channels [2, 3]. These two hydrophobic domains are important for the binding and the transport of ions. The first five TMS 1–5 are separated from the last four TMS 6–9 through a large hydrophilic intracellular loop of 550 amino acids, named the f loop (Intracellular domain between transmembrane segments 5 and 6 of the sodium calcium exchanger) (Fig. 1).

Although the f loop is not implicated in Na^+ and Ca^{2+} translocation, it is responsible for the regulation of NCX activity elicited by several cytoplasmic messengers and transductional mechanisms, such as Ca^{2+} , Na^+ and H^+ ions, NO (►nitergic systems, NO-synthases), phosphatidylinositol 4,5 bisphosphate (PIP2), protein kinase C (PKC), protein kinase A (PKA), and ATP. Furthermore, in the f loop, there are two Ca^{2+} binding domains named Ca^{2+} binding domain 1 (CBD1) [2, 3], containing an immunoglobulin-like fold with four Ca^{2+} ions bound and Ca^{2+} binding domain 2 (CBD2). CBD1 is the primary Ca^{2+} sensor and detects slightest increases in

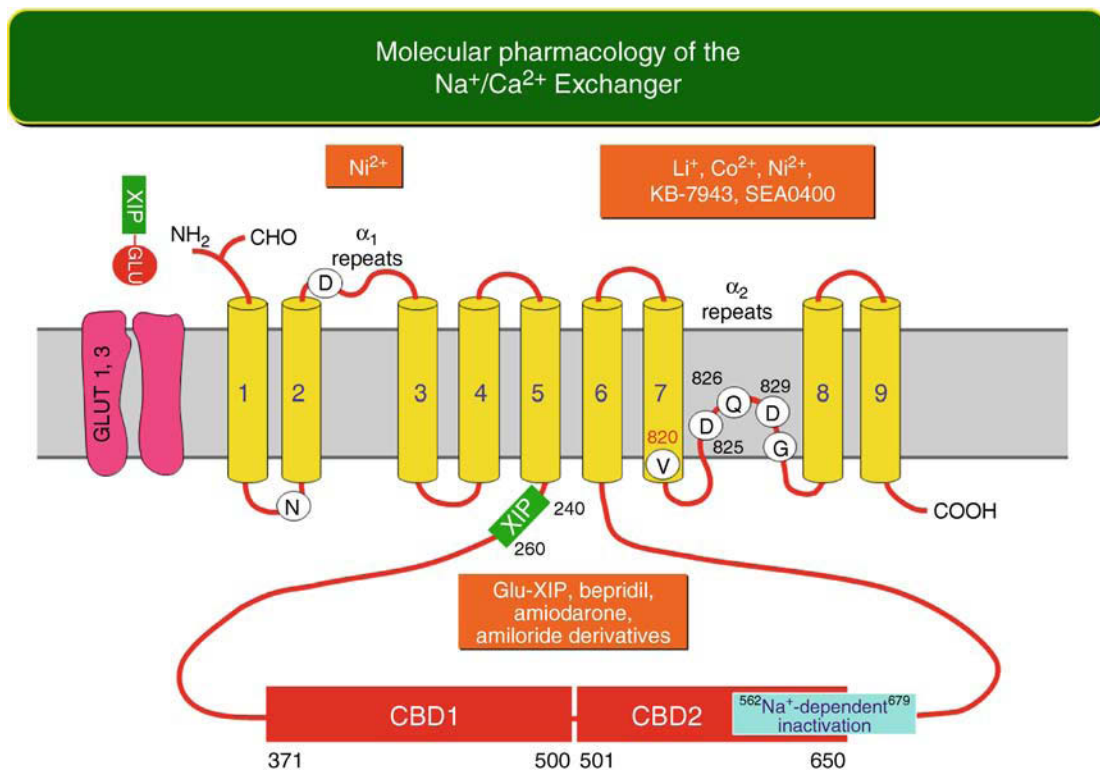
cytosolic Ca^{2+} and the associated large structural changes activate the exchanger. In contrast, CBD2 undergoes comparably modest structural alterations and binds Ca^{2+} only at elevated Ca^{2+} concentrations. These two different sensitivity thresholds may enable NCX to function dynamically over a wide range of Ca^{2+} concentrations and permit high Ca^{2+} fluxes in excitable cells.

At the N-terminal end of the f loop, near the membrane lipid interface, there is an autoinhibitory domain, rich in both basic and hydrophobic residues and consisting of a 20-aminoacid sequence (219–238). This aminoacid sequence, named exchange inhibitory peptide (XIP), is involved in NCX activity regulation.

The other two isoforms NCX2 and NCX3 have a molecular topology similar to NCX1. They consist of 921 and 927 amino acids and are characterized by molecular masses of 102 and 105 kDa, respectively. NCX2 displays a 65% sequence identity with NCX1, whereas NCX3 possesses a 73% sequence identity with NCX1 and 75% sequence identity with NCX2. All three NCX gene products share the same membrane topology.

Distribution

$\text{Na}^+/\text{Ca}^{2+}$ exchanger activity is present in virtually every cell type examined. The NCX1 gene is expressed in several tissues, including brain, heart, skeletal



$\text{Na}^+/\text{Ca}^{2+}$ Exchangers. Figure 1 Molecular pharmacology of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Topology and putative sites of the molecular action of drugs interfering with NCX activity (Reproduced from Annunziato L, Pignataro G, Di Renzo GF (2004) *Pharmacol Rev* 56:633–654).

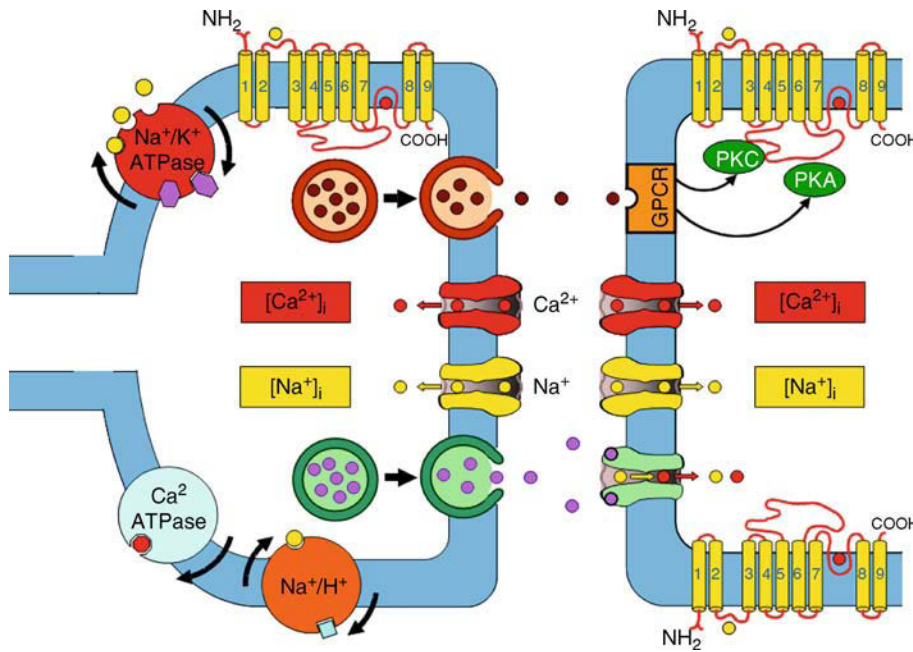
muscle, smooth muscle, kidney, eye, secretory, immune system, and blood cells, whereas transcripts and proteins encoded by the NCX2 and NCX3 genes have been found exclusively in neuronal and skeletal muscle tissues. In addition, NCX1 and NCX3 give rise to several splicing variants that appear to be selectively expressed in different regions and cellular populations of the brain (Fig. 2).

Regulatory Factors

Several factors are involved in the regulation of Na⁺/Ca²⁺ exchanger activity: (i) the two transported cations, Na⁺ and Ca²⁺; (ii) the intracellular pH; (iii) the metabolic related compounds, ATP, PIP₂, PKA, and PKC; (iv) reactive oxygen species.

The site level at which [Ca²⁺]_i regulates NCX activity (CBD) is different from the one required for Ca²⁺ transport. Submicromolar concentrations (0.1–0.3 μM) of intracellular Ca²⁺ are needed to activate the antiporter through these Ca²⁺ binding site. The location of such regulatory site has been identified in the 134-amino acid-length region, situated in the center of the intracellular f loop [2]. (Table 1)

An increase in [Na⁺]_i can also regulate the Na⁺/Ca²⁺ exchanger. In particular, when intracellular Na⁺ increases, it binds to the transport site of the exchanger molecule, and after this Na⁺ influx, an inactivation process of the exchanger occurs. This inactivation process, very similar to the phenomenon occurring in voltage-dependent ionic channels, is named



Na⁺/Ca²⁺ Exchangers. Figure 2 Schematic diagram of NCX localization and function in a pre- and postsynaptic structure (Reproduced from Annunziato L, Pignataro G, Di Renzo GF (2004) Pharmacol Rev 56:633–654).

Na⁺/Ca²⁺ Exchangers. Table 1 Regulatory mechanisms of Na⁺/Ca²⁺ exchanger activity

Regulatory Factors	Effect
[Ca ²⁺] _i	Increase Na ⁺ /Ca ²⁺ exchanger activity (Low affinity: 0.1–0.3 μM; high affinity: 20–50 nM)
[Na ⁺] _i	[Na ⁺] _i -dependent inactivation of outward Ca ²⁺ currents
NO	Stimulates Na ⁺ /Ca ²⁺ exchanger activity via cGMP
Redox Status	Oxidizing agents stimulate activity
PIP ₂	Elimination of the [Na ⁺] _i -dependent inactivation
ATP	↑ [Na ⁺] _o -dependent Ca ²⁺ efflux
PKC	Increases the affinity of [Ca ²⁺] _i and [Na ⁺] _o for the Na ⁺ /Ca ²⁺ exchanger
PKA	↑ Activity in exon A-carrying splicing isoforms of the Na ⁺ /Ca ²⁺ exchanger
PKT	↑ Expression and activity of NCX1 and NCX3

Na^+ -dependent inactivation. There is a sequence of 20-aminoacids (219–238), located in the intracellular f loop near the membrane lipid interface, that seems to be involved in the Na^+ -dependent inactivation. This autoinhibitory 20-aminoacid sequence might interact with another portion of the f loop (562–679) producing NCX inhibition. In accordance with this view, the synthetic peptide provided with the same sequence of XIP region blocks NCX activity.

The intracellular pH can also regulate the exchanger. $[\text{H}^+]$ strongly inhibits NCX activity under steady-state conditions, in fact, reduction in $[\text{pH}]_i$ values, as little as 0.4, can induce a 90% inhibition of NCX activity. Such inhibitory action depends on the presence of intracellular Na^+ ions, hence, the action exerted by H^+ ions is pathophysiologically relevant with regards to brain and heart ischemia.

ATP, acting as a phosphoryl donor molecule, may increase the activity of the exchanger in a number of ways. Firstly, ATP directly participates in the NCX molecule phosphorylation process by PKA and PKC. Secondly, it increases PIP2 production. Finally, by activating G-protein-coupled receptors, via endogenous and exogenous ligands, ATP can stimulate the activity of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger through the pathway involving PKC or PKA activation. The mechanism underlying the phosphorylating effect on the exchanger is related to an increase in its affinity for both internal Ca^{2+} and external Na^+ and to a decrease in its inhibition by internal Na^+ . Each of the NCX isoforms has distinctive putative phosphorylation sites; ATP cellular depletion inhibits NCX1 and NCX2 but does not affect NCX3 activity. The other mechanism by which ATP can activate NCX occurs through the production of the lipid PIP2. In fact, this lipid binding the XIP region of the f loop eliminates NCX inactivation and stimulates NCX function. In addition, $\text{Na}^+/\text{Ca}^{2+}$ exchanger can be sensitive to reactive oxygen species, since modifications of the cellular redox state can cause an increase of NCX activity.

Pathophysiological Implications

In consideration of the relevant role played by the forward and reverse mode operation of NCX in the maintenance of $[\text{Na}^+]_i$ and $[\text{Ca}^{2+}]_i$ homeostasis in cells of the cardiovascular and central nervous system, alterations in gene structure, gene activation, protein expression, stability, and function may take part in the pathophysiology of several diseases. As matter of fact, homozygous overexpression of NCX1 in the heart results in mild cardiac hypertrophy and in a higher susceptibility to ischemia-reperfusion injury, whereas NCX1 gene deletion confers protection. In addition, NCX is widely recognized as a contributing factor to both impaired contractile performance and electrical instability like arrhythmia in human and experimental heart failure. It is

also hypothesized that in salt-dependent hypertension NCX1, operating in the reverse mode, may be involved in Ca^{2+} entry, thus resulting in vasoconstriction. Recently, a relevant role has been attributed to NCX during focal cerebral ischemia since a stimulation of NCX activity, promoting Ca^{2+} extrusion, may help neurons and glial cells that are not irreversibly damaged in the penumbral zone to survive, whereas antisense knocking-down of NCX1 and NCX3 transcripts, blocking produces a significant worsening of ischemic brain injury [4]. This evidence suggests that a pharmacological intervention aimed at increasing NCX expression and activity might be a promising perspective to reduce cerebral damage after ischemic insult.

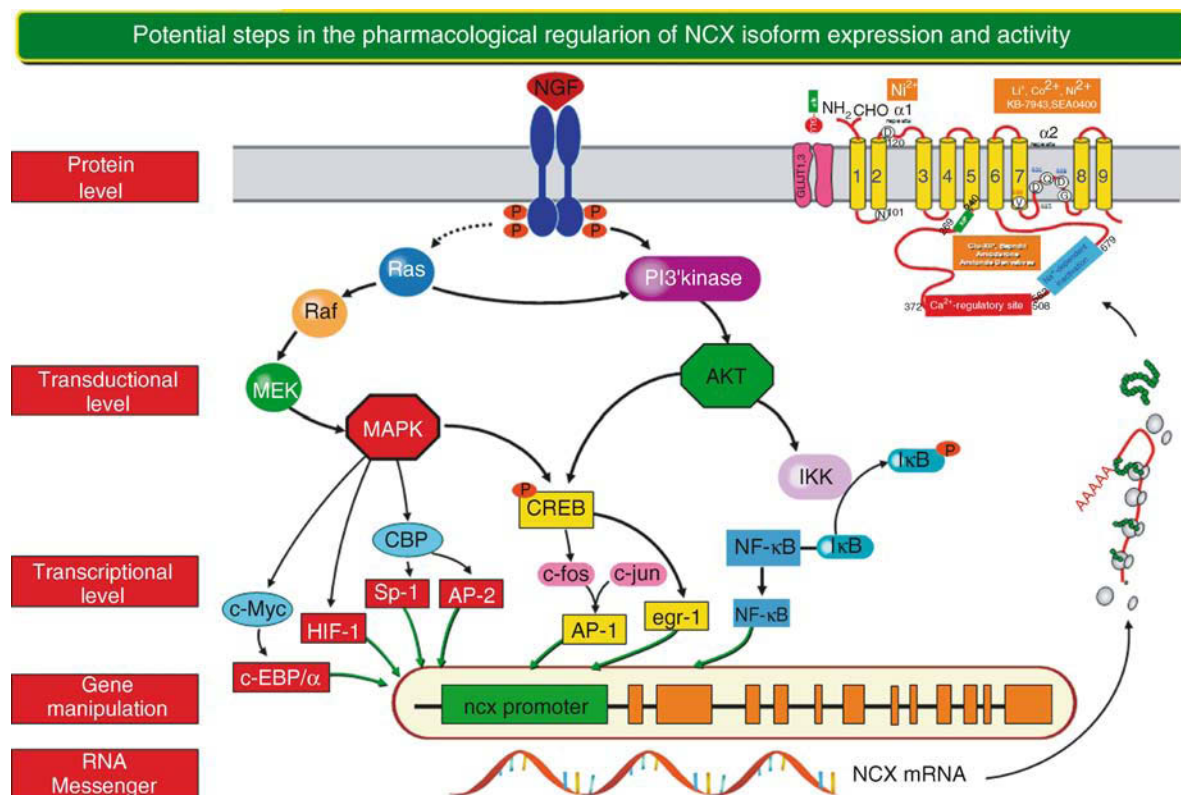
Drugs

Molecular Pharmacology of $\text{Na}^+/\text{Ca}^{2+}$ Exchanger

A great deal of interest has been devoted to the pharmacological modulation of NCX (Fig. 3). The reasons for this enormous interest lay in the hope of finding clinically effective drugs for those pathophysiological conditions such as cerebral ischemic disease, arrhythmias, heart failure, and hypertension in which a stimulation or an inhibition of the NCX might have achieved beneficial effects. In the last 35 years several inorganic and organic compounds have been reported to activate or to block NCX activity. (Table 2 and 3)

However, these compounds besides inhibiting NCX may also interfere with other cellular ion transporting mechanisms, i.e., Na^+ epithelial channels; K^+ channels; plasma membrane store-operated Ca^{2+} channels; VGCC-, NMDA-, and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptor-operated channels; Na^+/H^+ exchanger; Na^+/K^+ ATPase; and plasma membrane Ca^{2+} ATPase. In addition, other aspects that should be taken in consideration in the pharmacological modulation of NCX activity are: (i) the existence of three different gene products NCX1, NCX2, and NCX3 that, although all share a high degree of aminoacid sequence homology, display a different pharmacological sensitivity; (ii) the existence of at least 15 splicing variants for NCX1 and 5 for NCX3, each of them might be selectivity modulated by various compounds; (iii) the double mode of operations of the exchanger, forward or reverse, that can be modulated in a differential manner by different drugs (Fig. 4). Thanks to site direct mutagenesis, a great advancement has been achieved in the last few years in the molecular pharmacology characterization of the domains, of the transmembrane segments, of the connecting loops, and finally of the single aminoacids responsible of drug action (Fig. 1).

Among the organic derivatives, amiloride analogs were the first to be described as powerful inhibitors of NCX activity when the antiporter operates either in the forward or the reverse mode of operation (Fig. 5). These



Na⁺/Ca²⁺ Exchangers. Figure 3 Potential steps in the pharmacological regulation of NCX isoform expression and activity. This scheme reproduces the potential levels at which drugs can interfere with the transduction, transcription, translation, and activity of NCX.

Na⁺/Ca²⁺ Exchangers. Table 2 Drug families inhibiting the Na⁺/Ca²⁺ exchanger

Pharmacological Class	Drugs
Amiloride derivatives	CB-DMB, DCB, DMB
Substituted pyrrolidines	Bepidil
Isothiourea derivatives	KB-R7943
Ethoxyanilines	SEA0400
Benzofuran derivatives	Amiodarone
Quinazolinone derivatives	SM-15811
Thiazolidine derivatives	SN-6
Phenoxy pyridine derivatives	JP11092454
Nicotinamide derivatives	YM-244769
Piperidine derivatives	YM-252077
Ylacetamide derivatives	YM-270951
Peptides	XIP, Glu-XIP, FMRFa, FRCRCF
Small interference RNA	siRNA-NCX1, siRNA-NCX1, siRNA-NCX3
Antisense oligodeoxynucleotides	AS-NCX1, AS-NCX2, AS-NCX3
Inorganic cations	Ni ²⁺ , La ²⁺ , Gd ²⁺

compounds are reversible blockers and the inhibition is competitive with respect to Na⁺ ions. The compounds of the amiloride family bearing substituents on the

terminal guanidino hydrogen atom behave as a specific NCX inhibitor but are devoided of any effect on other antiporters such as Na⁺/H⁺ exchanger.

$\text{Na}^+/\text{Ca}^{2+}$ Exchangers. Table 3 Compounds stimulating the $\text{Na}^+/\text{Ca}^{2+}$ exchanger

Pharmacological Class	Drugs
Redox agents	DTT, GSH, Fe^{2+} , O_2^- and of Fe^{3+} , H_2O_2
Organic compounds	Diethylpyrocarbonate (DEPC)
Inorganic cations	Li^+
Agonists of GPCRs	α , β Agonists, histamine, 5HT_{2c} agonists, endothelin-1, angiotensin-II
Peptides	Insulin, concanavalin A
Neurotrophic factors	NGF

Molecular properties of drugs interacting with NCX

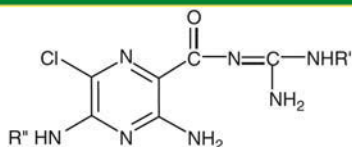
Drug	IC_{50} (μM)	Mode selectivity	Site of action	Site of action at Aminoacid level	Isoform specificity
Bepidil	8.1	Forward > reverse mode	f loop	—	—
CB-DMB	7.3	Forward, reverse mode	Na^+ binding site	—	$\text{NCX1} > \text{NCX3} > \text{NCX2}$
KB-R7943	1–2.5 > 30	Reverse > forward mode	α -2 repeat	Ala809, Val820, Gln826, Gly833, Asn839	$\text{NCX3} > \text{NCX2} > \text{NCX1}$
SEA0400	0.05–0.09	Reverse > forward mode	TMS5, α -2 repeat XIP region	Phe213, Gly833, Tyr224, Tyr226, Tyr228, Tyr231	$\text{NCX1} > \text{NCX2}$
SN-6	0.3–30	Reverse mode	TMS5, XIP region α -2 repeat	Phe213, Gly833 and Asn839, Val227 and Val238	$\text{NCX1} > \text{NCX3} > \text{NCX2}$
GLU-XIP	0.1–1	Forward, reverse mode	f loop	445–455	NCX1 , NCX2 , NCX3

 $\text{Na}^+/\text{Ca}^{2+}$ Exchangers. Figure 4 Molecular properties of drugs interacting with NCX. Scheme summarizing the potency, the mode of selectivity, the molecular site of action and the isoform specificity of drugs interfering with NCX activity.

More recently, Shigekawa's group screening a compound library for the inhibitions of Na^+ -dependent Ca^{2+} uptake identified an isothiourea derivative named KB-R7943 which, in the low micromolar range, is able to block the reverse mode operation of the antiporter, whereas concentrations 30 times higher are needed to inhibit the forward mode. Another peculiarity of this isothiourea compound is the different ability to block NCX activity depending on the gene product involved. In fact, NCX3 inhibition requires concentrations that are threefold lower than those necessary to inhibit NCX2 and NCX1. KB-R7943 interacts with α -2 repeat of the exchanger molecule at the level of Ala809, Val820, Gln826, Gly833, and Asn839. In light of these peculiar pharmacological properties, in the last 7 years, KB-R7943 has aroused a great deal of interest among investigators working on NCX activity. However, recent reports have shown that KB-R7943 also exerts an inhibitory effect on several other transport

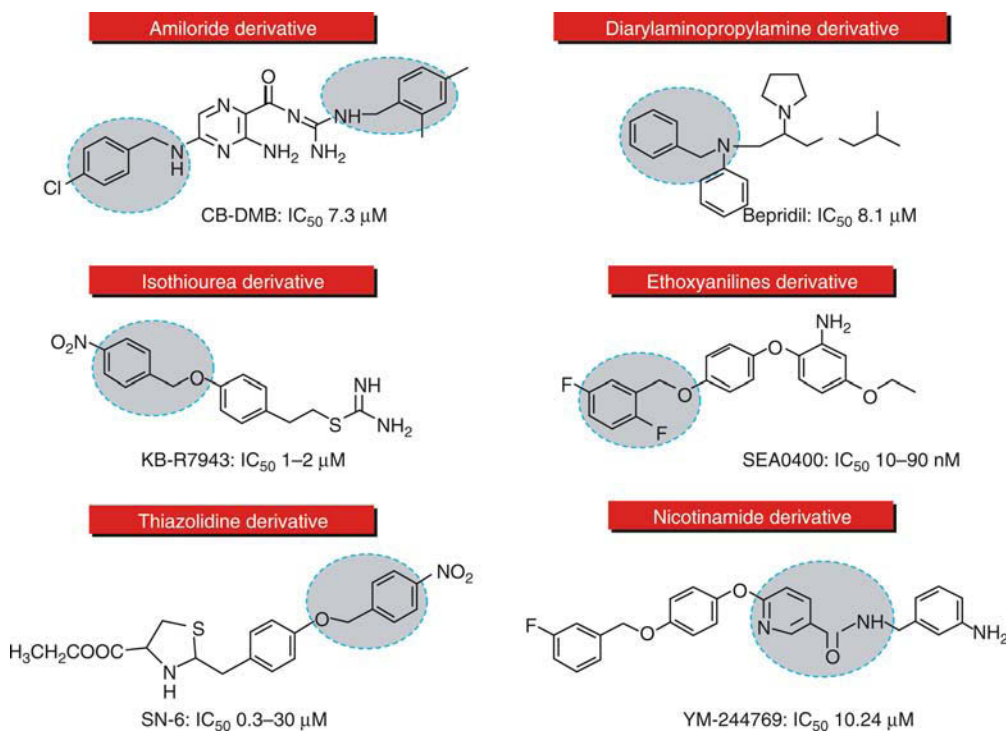
mechanisms such as L-type voltage gated Ca^{2+} channels, and receptor-operated NMDA channels (Fig. 6).

In 2001, a new compound belonging to the ethoxyanilines family, SEA0400, was reported as being the most potent NCX inhibitor available at the time, $\text{IC}_{50} = 5\text{--}92$ nM, with a predominant activity on NCX1, a lower affinity for NCX2 and no effect on NCX3. However, the specificity of SEA0400 on NCX activity has recently been questioned, since it can also interfere with Ca^{2+} movement across the cell membrane. In 2002, by screening benzyloxyphenyl derivatives, Iwamoto's group discovered the new compound SN-6, which differs from KB-R7943 only in the substituent of phenyl moiety. The presence of this phenyl group confers to this antiporter inhibitor a more selective action on NCX1 rather than NCX3. Using chimeric analysis and subsequent site-directed mutagenesis some critical aminoacid residues responsible of SN-6 inhibition in the XIP region of the antiporter have

Chemical structures of amiloride derivatives and their IC₅₀ on NCX and NHX activity

Drugs	R'	R''	NCX IC ₅₀ (μM)	NHX IC ₅₀ (μM)
Amiloride	-H	-H	1100	83.8
2',4'-dimethylbenzamil (DMB)		-H	10	63
α-phenylbenzamil (α-PB)		-H	115	>1000
5-[N-methyl-N-(guanidinocarbonylmethyl)] benzamil (MGCB)	-H		83	16
2',4'-Dimethyl-5-[N-(p-chlorobenzyl)]benzamil (CB-DMB)			7.3	>500

Na⁺/Ca²⁺ Exchangers. Figure 5 Chemical structures of amiloride derivatives and their IC₅₀ on NCX and NHX activity. Chemical structure of the two classes of amiloride derivatives and their inhibitory concentrations on NCX and Na⁺/H⁺ exchanger activity (Reproduced from Annunziato L, Pignataro G, Di Renzo GF (2004) Pharmacol Rev 56:633–654).



Na⁺/Ca²⁺ Exchangers. Figure 6 Chemical structures of some NCX inhibitors. IC₅₀ values for NCX inhibition of drugs belonging to different chemical families.

Na⁺/Ca²⁺ Exchangers. Table 4 Oligodeoxynucleotide sequences

Target gene	Sequence
NCX1	5'-TGAGACTTCCAATTGTT-3'
NCX2	5'-TCGGAGTAGCTTC-3'
NCX3	5'-GCCATACACAAGAC-3'

been identified. Interestingly, SN-6 preferentially acts on the exchanger under ATP-depleted conditions [5].

Very recently, the further screening of new benzyloxyphenyl derivatives revealed a highly potent NCX inhibitor, named YM-244769. This orally bioavailable compound is more potent inhibiting NCX3 than NCX1 and NCX2 in the reverse mode, but it is not active on the forward mode of operation of the three antiporter isoforms. (Table 4)

Recently, two more selective strategies able to inhibit each of the three NCX gene products, has been introduced as pharmacological tool. These strategies consist in the antisense oligodeoxynucleotide technique and small interference RNA directed against mRNA sequence of NCX1, NCX2, and NCX3. With this potentially therapeutic approach it has been possible to discriminate the role played by each of the three NCX gene products in preclinical models of pathological states in which this antiporter is involved.

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NaCl Cotransporter

The thiazide sensitive NaCl cotransporter (NCC) is the major pathway of NaCl entry in the distal convoluted tubule. Like NKCC2, NCC contains 12 putative transmembrane domains and long intracellular amino- and carboxy-tails. NCC and NKCC as well as the KCl cotransporter KCC are members of the same gene family and have considerable homology.

► Diuretics

Na⁺-dependent Glucose Cotransporter

Synonyms

SGLT

Definition

Sodium-dependent glucose membrane-spanning (SGLT) transport glucose into the cell against its concentration gradient. These transporters catalyze intestinal glucose adsorption, and renal reabsorption. SGLT1 is a high-affinity transporter present in both intestinal brush-border membranes and renal proximal tubules. The Na⁺/sugar stoichiometry of SGLT1 is 2:1. SGLT2 is a low-affinity transporter expressed in the S1 segment of the early proximal tubules of the kidney. SGLT2 exhibits a Na⁺/glucose stoichiometry of 1:1. SGLT3 is a low-affinity transporters expressed in both kidney and intestine. The Na⁺/glucose stoichiometry of SLGT3 is 2:1.

► Glucose Transporters

Na⁺-dependent Glucose Cotransporter

Sodium-dependent glucose cotransporters (SGLT) transport glucose into the cell against its concentration gradient. These transporters catalyze intestinal glucose adsorption and renal re-absorption. SGLT1 is a high-affinity transporter present in both intestinal brush border membranes and renal proximal tubules. The

Na⁺ Channel

- Voltage-dependent Na⁺ Channels
- Epithelial Na⁺ Channels

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► Glucose Transporters

Na⁺/H⁺ Exchangers

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Synonyms

Sodium–proton exchanger; Sodium–proton antiport

Definition

Sodium–hydrogen ion exchangers (►NHEs) are a family of membrane proteins present in most living organisms, from simple prokaryotes to complex eukaryotes. They mediate the exchange of protons for sodium ions across the plasma membrane and possibly also across organellar membranes, and thereby play a role in the regulation of pH homeostasis, cell volume and transepithelial sodium transport. Not surprisingly, Na⁺/H⁺ exchangers are implicated in many pathological processes including hypertension, congenital secretory diarrhoea and ischemia-reperfusion injury. Within the human genome there are nine distinct genes (SLC9A1–9) encoding sodium–proton exchangers [1]. The proteins are made up of ~800 amino acid residues and share a highly homologous amino-terminal transmembrane domain, predicted to be composed of 12 transmembrane helices, and a more divergent cytoplasmic carboxy terminus, as shown in Fig. 1. There is between 20% and 70% homology amongst the various exchanger isoforms.

Basic Characteristics

The exchange of cations for protons occurs through the transmembrane domain, whereas regulation (fine-tuning) of the exchanger is largely exerted by the C-terminal domain. NHEs are believed to form homodimers in the membrane.

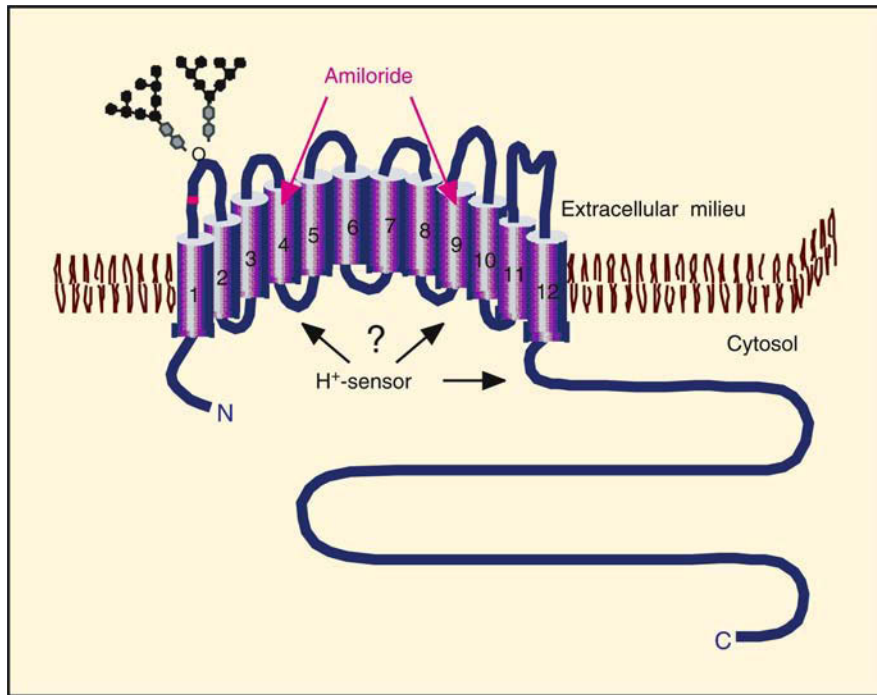
The exchange of sodium ions for protons occurs with a 1:1 stoichiometry, and is thus an electroneutral event. Transport is a passive, energetically downhill event, not

requiring direct investment of metabolic energy. Nevertheless, physiological levels of ATP are required for optimal function of the exchanger. In its normal physiological (forward) mode, the process is driven by the inward sodium gradient, which is generated and maintained by the ►Na⁺-K⁺-ATPase (Na⁺-K⁺ pump), an ATP-dependent enzyme. Plasmalemmal NHEs can also translocate other monovalent cations such as lithium and ammonium, though at a slower rate. Potassium is not transported measurably by the plasmalemmal exchangers, but the organellar exchangers may utilise this cation, as suggested for isoform NHE7. Kinetically, NHEs obey simple Michaelis–Menten kinetics, displaying a hyperbolic dependence on extracellular sodium concentration, indicative of a single binding site for sodium. In contrast, they show greater-than-first-order dependence on the intracellular hydrogen ion concentration, suggesting allosteric regulation. Indeed, it is generally believed that NHEs possess an intracellular allosteric modifier site responsive to protons, in addition to the proton transport site. This type of allosteric regulation has been documented only for a couple of the plasmalemmal NHEs and may not apply to all the isoforms.

The activity of the NHEs is markedly reduced when ATP levels are depleted, even if the sodium gradient is maintained. Truncation mutants of NHE1 in which all the known phosphorylation sites are removed, still retain at least a partial responsiveness to ATP depletion. This can be explained in part by the dependence and likely association of NHE1 with phosphatidylinositol 4,5-bisphosphate (►PIP₂), a plasmalemmal lipid. Upon ATP depletion, PIP₂ is gradually depleted, resulting in decreased NHE1 activity. This type of regulation may not apply to all isoforms, several of which reside in membranes with undetectable low levels of PIP₂.

NHE1. This isoform was identified in 1989 and because of its ubiquitous distribution is thought to perform ‘housekeeping’ functions, maintaining intracellular pH and cell volume homeostasis [2]. NHE1 is found on the plasma membrane of virtually all mammalian cells, where it can seemingly accumulate in microdomains. In some cells, however, NHE1 is not present throughout the entire cell surface, reflecting a more specialised role for this isoform in particular cell types, such as epithelia. Thus, NHE1 activity is required for the secretory function of parotid acinar cells. In addition to its housekeeping and secretory roles, NHE1 has been suggested to regulate cell proliferation, migration and adhesion, and to confer resistance to apoptosis. These extra properties of NHE1 may be achieved through remodelling of the cortical actin cytoskeleton. In this regard, NHE1 is known to interact with proteins of the ERM (ezrin, radixin and moesin) family.

NHE1-deficient mice survive into the postnatal period but develop growth retardation, progressive



Na^+/H^+ Exchangers. Figure 1 Prototypical structure and transmembrane disposition of NHE. The amino (N) and carboxy (C) termini of the molecule are indicated.

neurodegeneration with ataxia and seizures, and have a high mortality before weaning. This phenotype is associated with a reduced steady-state intracellular pH and hyperexcitability of hippocampal neurons, but the precise molecular mechanisms responsible for the phenotype are as yet unknown.

Acute over-activation of NHE1 results in a marked elevation in intracellular sodium concentration with a subsequent increase in intracellular calcium, via the $\text{Na}^+/\text{Ca}^{++}$ exchanger. This in turn triggers a cascade of injurious events that can culminate in tissue dysfunction and ultimately apoptosis and necrosis. This is commonly seen in organs such as the heart, brain and kidneys as a consequence of ischemia-reperfusion.

The regulation of NHE1 is complex and involves multiple growth factors and hormones [3]. Phosphorylation of NHE1 by various protein kinases, including Erk 1/2, p160ROCK and p38 alters the set-point of the allosteric modifier site, enabling the exchangers to remain active at more alkaline pH. In addition to phosphorylation, NHE1 activity is regulated by association with ancillary proteins. In this manner, calmodulin and carbonic anhydrase II stimulate NHE1. The calcineurin B homolog proteins (CHP) also regulate NHE1. The CHP1 isoform is felt to be inhibitory, while CHP2 activates NHE1, which is required for optimal exchanger activity. CHP proteins are constitutively phosphorylated and associated with NHE1. Their dephosphorylation can

result in disassociation from the exchanger, with accompanying changes in exchange activity.

NHE2. NHE2 is found predominantly in the ►apical membrane of epithelial cells of the gastro-intestinal tract and also in the kidney, especially in the tubules. Although it regulates bicarbonate excretion in the distal convoluted tubule of the kidney, this isoform appears to contribute mainly to the secretory function of certain glands. NHE2 knockout mice have decreased parotid gland secretion plus a significant loss of gastric parietal and zymogenic cells, but no apparent intestinal or renal absorptive defects.

The regulation of NHE2 is multifactorial. Chronic exposure to nitric oxide and gamma-interferon decrease NHE2 activity, whereas metabolic acidosis and chronic stimulation with epidermal growth factor (EGF) increase activity.

NHE3. This isoform, with NHE1, has been most extensively studied. NHE3 is found mainly in the apical membrane of renal tubular epithelial cells, particularly those of the proximal tubule, and also in the gut. Of note, this isoform is not detectable in the basolateral membrane. In addition to its presence in the apical plasma membrane, NHE3 is found also in subapical vesicles that are thought to traffic to and from the membrane, thereby contributing to the acute regulation of exchange activity by altering the density of surface-exposed exchangers. Proximal tubular NHE3 is

responsible for approximately two thirds of sodium reabsorption in the kidney and contributes significantly to the ability of the kidney to concentrate urine. NHE3-deficient (knockout) mice have diarrhoea, alkalinised intestinal contents, increased urine output, mild acidosis, reduced blood pressure and elevated serum aldosterone levels. A rare, but severe, congenital secretory diarrhoea is believed to be due to decreased NHE3 activity in the intestinal brush-border in humans. Abnormal NHE3 activity is also implicated in the development of hypertension, though the exact pathophysiology is not yet known. Similarly the role of NHE3 in renal ischemia-reperfusion injury has still to be fully established.

Regulation of NHE3 involves many hormonal and physical mechanisms. Acutely, NHE3 activity is promoted by an increase in intracellular pH and this response is rapid. Furthermore, the increase in activity is proportional to the duration of acidification. Chronic acidification promotes recycling of NHE3 from subapical endosomes to the plasma membrane.

NHE3 is phosphorylated in the basal state. There are phosphorylation sites for both ►protein kinase A (PKA) and ►protein kinase C (PKC). Phosphorylation brings about a change in the rate of traffic between the subapical endosomes and the plasma membrane. However, acute changes in NHE3 activity can occur without changes in the surface expression of NHE3. Cyclic AMP (cAMP) for example inhibits NHE3 activity without reducing the surface expression of the exchanger. Parathyroid hormone and dopamine exert their effects by increasing cAMP.

NHE3 is also regulated by accessory proteins. Na⁺/H⁺ exchanger regulatory factors 1 and 2 (NHERF 1 and 2) are proteins that are believed to tether NHE3 to the actin cytoskeleton via an additional linker protein, namely ezrin. Unlike NHE1, disruption of the actin cytoskeleton decreases NHE3 activity and this is, at least in part, mediated by the small GTP-binding protein, RhoA, which is also a major organiser of the actin cytoskeleton. As in the case of NHE1, proteins that bind to regions of the cytoplasmic tail such as CHP1, also regulate NHE3. A role of lipid rafts in the acute regulation of NHE3 has been proposed.

Chronic up-regulation of NHE3 is stimulated by glucocorticosteroids, aldosterone, thyroxine, butyrate and chronic acidosis, amongst others. These factors stimulate transcription of the NHE3 gene. Less well documented are the mechanisms involved in chronic down-regulation of NHE3, though gamma-interferon is known to inhibit exchanger activity and this may account for its effect in inflammatory bowel disease.

NHE4. NHE4 is also located in the epithelial cells of the kidney and gut, but is seemingly basolateral rather

than apical. Little is known regarding the role and regulation of this isoform but it probably has some functional overlap with NHE1. Knockout mice have no obvious phenotype, except for a slightly decreased gastric acid secretion and an abnormal-looking gastric mucosa on biopsy. The effect of NHE4 ablation on kidney function has not been firmly established.

NHE5. The distribution of this isoform is distinct, being in neuronal-rich areas of the central nervous system. Low levels have also been found in testis, spleen and skeletal muscle. Like the preceding isoforms, NHE5 is found in the plasma membrane and is internalised by clathrin-associated endocytosis into recycling endosomes. The normal role of NHE5 is unknown but its malfunction is speculated to contribute to the development of neurodegenerative disease.

Stimulation of PKA and PKC inhibit NHE5 activity, as does hyperosmolarity, resembling the responsiveness of NHE3. NHE5 may also be regulated by the actin cytoskeleton, but further work is required to validate this notion and clarify the exact mechanism.

NHE6–9. NHEs 6–9 are all intracellular exchangers and they are distributed ubiquitously, though NHE8 differs slightly from the others in that it has higher levels in skeletal muscle and kidney. NHE6 is located in early and recycling endosomes, NHE7 in the trans-Golgi network, NHE8 in the mid- and trans-Golgi stacks and NHE9 in recycling endosomes [4]. These isoforms are thought to contribute to the maintenance of organellar pH and volume homeostasis, but little direct evidence exists for these proposed functions. Virtually nothing is known about proteins that associate with the endomembrane NHEs and whether they or other factors contribute to regulation of ion exchange.

Drugs

The first inhibitor of NHE, ►amiloride, was identified in 1982. This drug is a potassium-sparing diuretic that also inhibits the sodium–calcium exchanger and the conductive Na⁺ channel. Not all the NHE isoforms are inhibited equally by amiloride: NHE1 and 2 are responsive, NHE5 is partially responsive and NHE3, 4 and 7 are resistant. Other weak and non-specific inhibitors are clonidine and cimetidine.

Inhibition of NHE1 offered the potential to attenuate cardiac damage, particularly during ischemia-reperfusion and cardiac failure and, with this aim, several new molecules were derived from amiloride. By substituting the nitrogen of the 5-amino group, pyrazines were created that are more potent than amiloride, and although they are more selective for NHE1, they still have some effect on other isoforms. These drugs include DMA, EIPA, MIBA and HMA [5].

Further modifications of amiloride, replacing the pyrazine ring with a phenyl moiety, the 6-chloro with a sulfomethyl group and the 2-amino with a methyl group, or deleting it completely, has resulted in the generation of the bezoylguanidines, such as HOE-694, ►cariporide (HOE-642), eniporide and BIIB-513. These compounds have no effect on the $\text{Na}^+/\text{Ca}^{++}$ exchanger or the Na^+ channels. They are more selective for NHE1 and have no effect on NHE3 or NHE5.

Thereafter, molecules have been synthesised with a bicyclic ring, such as a quinoline or an indole, inserted. Many of these compounds like zoniporide and BMS-284640 are selective NHE1 inhibitors, but some inhibit also other isoforms. Most recently, an additional group of compounds with 4-substituted (benzo[b]thiophene-2-carbonyl) guanidines has been synthesised and these are potent NHE1 inhibitors. A structurally distinct compound, S-3226, was found to be the first selective NHE3 inhibitor.

The potency of the inhibitors is affected by the pH. Changes in pH affect the protonation state of the guanidine. In conditions of low pH, such as in ischemia-reperfusion, some drugs such as cariporide work more efficiently because they are on average more positively charged.

Animal studies on the effects of these drugs in ischemia-reperfusion have been very promising and this has led to a rapid progression from animal studies to human trials of the drugs. Unfortunately the drugs have been less effective in large-scale human trials, particularly after a myocardial infarction. This may be due to administration of the drug too late in the pathological process. It seems that NHE1 inhibition is most cardio-protective in the ischemia stage and, when drugs are administered post-infarction the ongoing reperfusion causes damage that they cannot effectively counteract. Supporting this concept are the better results obtained in patients undergoing coronary artery bypass graft surgery. When the inhibitor was administered pre-operatively, fewer patients went on to have myocardial infarction compared to those who received placebo. There is currently a large trial underway to further assess this effect. The use of NHE1 inhibitors in heart failure is also currently under evaluation. Animal studies also suggest a beneficial role for NHE1 inhibition in the prevention of cerebral ischemia-reperfusion injury.

Evaluation of inhibition of the other isoforms is still in the preclinical stages. Inhibition of NHE2 with prostaglandin E2 or with amiloride hastens the re-establishment of barrier function after intestinal ischemic damage in pigs and the use of S-3226 in rats with renal ischemia improved outcomes. Further work examining the effect of inhibition of the other isoforms is still required.

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Na^+ - and K^+ activated Adenosine 5'-Triphosphatase

► Na^+/K^+ -ATPase

Na^+/K^+ -ATPase

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Synonyms

Na^+/K^+ -ATPase; sodium pump; Sodium- and potassium-activated adenosine 5'-triphosphatase; EC 3.6.1.37.

Definition

The Na^+/K^+ -ATPase is an integral protein of the plasma membrane of animal cells that transports Na^+ and K^+ ions against their electrochemical gradients energized by ATP hydrolysis. For every ATP molecule hydrolyzed the Na^+/K^+ -ATPase moves 3 Na^+ ions from the cytosol to the extracellular space in exchange for 2 K^+ ions that are taken up from the extracellular medium. This uneven transport of positive charge – 3 out versus 2 in – is coupled with various elementary functions of the cell: (i) It substantially contributes to the formation and maintenance of the membrane potential of the cell, (ii) It contributes to the osmotic regulation of the cell volume, (iii) In higher organisms it provides the basis for neuronal communication, and (iv) It provides in all animal cells the driving force for the Na^+ gradient-coupled secondary active transport systems.

Basic Characteristics

The Subunit Composition of Na⁺/K⁺-ATPase

The Na⁺/K⁺-ATPase belongs to the P-type ATPases, a family of more than 50 enzymes that also includes the Ca²⁺-ATPase of the sarcoplasmic reticulum or the gastric H⁺/K⁺-ATPase. ►P-Type ATPases have in common that during ion transport an aspartyl phosphointermediate is formed by transfer of the γ -phosphate group of ATP to the highly conserved sequence DKTGS/T [1].

The Na⁺/K⁺-ATPase is an oligomeric protein that consists of α and β subunits. Four different isoforms of the α subunit ($\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 4$) have been identified with relative molecular masses varying between 100 and 113 kDa. They cross the membrane 10 times, forming transmembrane domains M1 to M10, and both amino and carboxy termini are localized on the cytosolic side [1]. The three β subunit isoforms ($\beta 1$, $\beta 2$, and $\beta 3$) identified thus far are highly glycosylated proteins of about 60 kDa, with the mass of the protein moiety ranging from 36–38 kDa. The β subunit crosses the membrane only once, and the amino terminus is localized on the intracellular side of the membrane. Next to the α and β subunits, which are absolutely required for catalytic activity, a third cytosolic peptide of 7–11 kDa, referred to as the γ subunit, appears in some tissues to be involved in regulating the activity of Na⁺/K⁺-ATPase by influencing its interactions with ATP, ouabain, and Na⁺ or K⁺ ions. The quaternary structure Na⁺/K⁺-ATPase with all three subunits has been recently resolved [2].

Na⁺/K⁺-ATPase Acts as a Sodium-Potassium Pump

The catalytic cycle of the Na⁺/K⁺-ATPase can be described by juxtaposition of distinct reaction sequences that are associated with two different conformational states termed E₁ and E₂ [1]. In the first step, the E₁ conformation is that the enzyme binds Na⁺ and ATP with very high affinity (K_D values of 0.19–0.26 mM and 0.1–0.2 μ M, respectively) (Fig. 1A, Step 1). After autophosphorylation by ATP at the aspartic acid within the sequence DKTGS/T the enzyme occludes the 3 Na⁺ ions (E₁-P(3Na⁺); Fig. 1a, Step 2) and releases them into the extracellular space after attaining the E₂-P 3Na⁺ conformation characterized by low affinity for Na⁺ ($K_{0.5}$ = 14 mM) (Fig. 1a, Step 3). The following E₂-P conformation binds 2 K⁺ ions with high affinity (K_D approx. 0.1 mM; Fig. 1a, Step 4). The binding of K⁺ to the enzyme induces a spontaneous dephosphorylation of the E₂-P conformation and leads to the occlusion of 2 K⁺ ions (E₂(2K⁺); Fig. 1a, Step 5). Intracellular ATP increases the extent of the release of K⁺ from the E₂(2K⁺) conformation (Fig. 1a, Step 6) and thereby also the return of the E₂(2K⁺) conformation to the E₁ATPNa conformation. The affinity of the E₂(2K⁺) conformation for ATP, with a $K_{0.5}$ value of 0.45 mM, is very low.

Drugs

The Na⁺/K⁺-ATPase as the Molecular Target for Cardiotonic Steroids (CTS) and Palytoxin

A vast number of toxins that specifically interact with the Na⁺/K⁺-ATPase and inhibit its catalytic activity have been isolated from various plants and animals (Fig. 2). The biggest group is constituted by substances referred to as cardiotonic steroids (CTS; also named cardiac glycosides or cardiac steroids) since their application, especially of digitalis and its congeners, help to treat chronic heart failure (NYHA II and IV), tachycardia, and dilatative cardiomyopathy. Treatment with low concentrations of digoxin significantly reduces mortality and hospitalization of patients with heart failure. This effect is believed to be due to a partial inhibition of Na⁺/K⁺-ATPase (Na⁺-lag hypothesis), possibly by indirectly inducing an elevation in the Ca²⁺ concentration in the myocardium. Nevertheless, other mechanisms are probable (see below).

The Na⁺/K⁺-ATPase is the only enzyme known to interact with CTS, which reversibly bind to the extracellular side of the Na⁺/K⁺-ATPase at the E₂-P conformational state [E₂-P* ouabain] and inhibit ATP hydrolysis and ion transport (Fig. 1b, step 4).

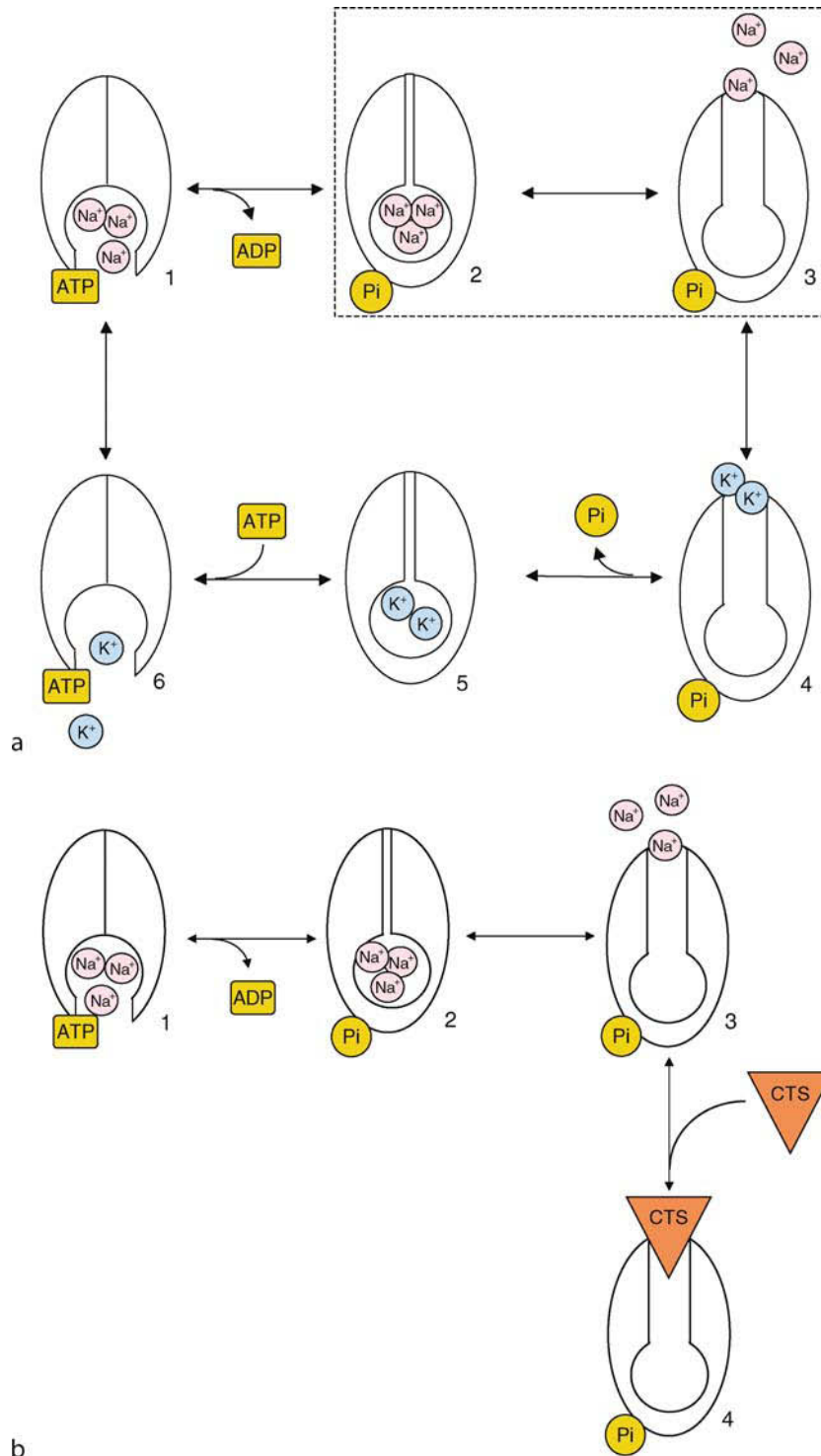
Besides CTS, the highly toxic palytoxin (LD₅₀ for rodents is 10–250 ng/kg), produced by corals of the genus *Palythoa*, is also a highly specific inhibitor of Na⁺/K⁺-ATPase (Fig. 2) [1]. Unlike the CTS, however, which stop ATP hydrolysis and ion flow, palytoxin, by binding to either the E₁ or the E₁-P conformation (Fig. 1c, steps 1 or 2, respectively), converts the sodium pump into an ion channel with a conductance of approximately 10 pS by arresting its natural ion-conducting structure in a permanently open conformation that allows K⁺ to flow down its concentration gradient out of the cell (Fig. 1c, step 3). Thus, the reason for the high toxicity of palytoxin might be associated with the outflow of K⁺, the collapse of the membrane potential, and the ensuing loss of basic cell functions. In addition, depolarization is a key event that affects numerous secondary systems. Thus, the concentration of Ca²⁺ becomes elevated in several organs through the opening of Ca²⁺ channels and leads to the production of inositol trisphosphate, the activation of phospholipase A₂ and metabolism of arachidonic acid, and numerous other physiological responses that all stem from the increased Na⁺ influx and the ensuing increase in the concentration of cytosolic Ca²⁺ that accompany the initial K⁺ outflow. The strong tumor-promoter activity of palytoxin might be associated with these events [3].

Na⁺/K⁺-ATPase Acts as Signal Transducer of Endogenous Cardiac Glycosides

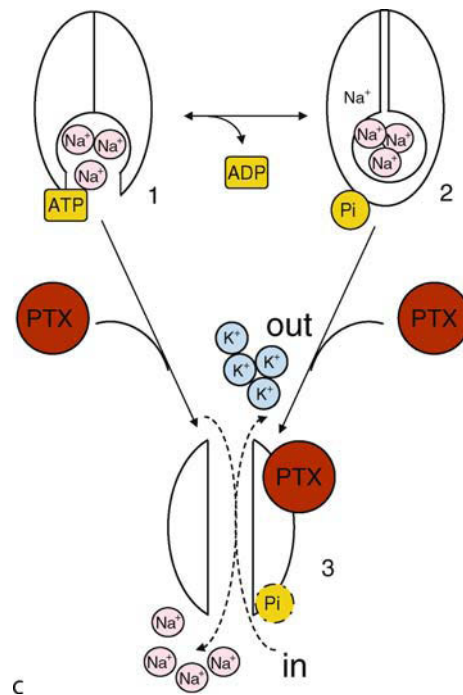
The most popular assumption for the action of cardiac glycosides is that an inhibition or partial inhibition may induce inotropy of the heart by raising intracellular

cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) via a coupling of $\text{Na}^+/\text{K}^+\text{-ATPase}$ with the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX; Fig. 3). This Na^+ lag hypothesis has been recently modified: It is assumed that an inhibition of the sodium pump by

cardiac glycosides may lead to a local increase of Na^+ in a tiny reactive space between the plasmalemma and the endoplasmic/sarco-plasmic reticulum (ER/SR) in smooth muscle cells and astrocytes, which has been



Na⁺/K⁺-ATPase. Figure 1 (Continued)

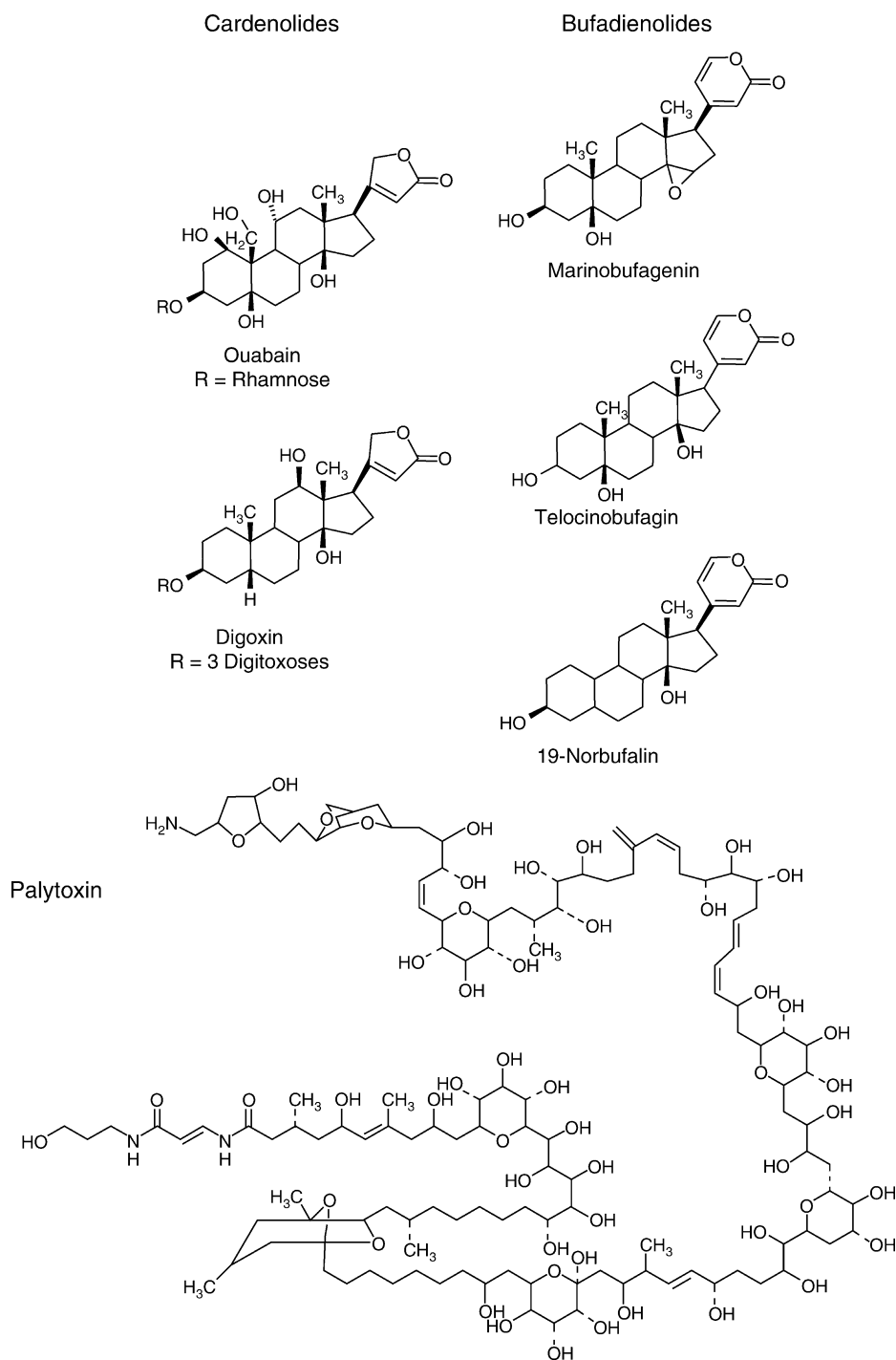


Na⁺/K⁺-ATPase. Figure 1 (a) Reaction cycle of Na⁺/K⁺-ATPase. The enzyme binds Na⁺ and ATP in the E₁ conformational state (Step 1) and becomes phosphorylated at an aspartate residue by the γ-phosphate of ATP. This results in occlusion of 3 Na⁺ ions (Step 2) followed by their release to the extracellular space (Step 3). This new conformational state, termed E₂-P, with low affinity for Na⁺ and high affinity for K⁺ binds K⁺ (Step 4) and occludes the cation after dephosphorylation (Step 5). Binding of ATP with low affinity to the E₂(2K⁺) state leads to K⁺ being released to the cytosol (Step 6). The dashed box highlights the electrogenic steps of the catalytic cycle. (b) Binding of CTS to the sodium pump. CTS bind preferentially to the E₂-P conformation of the enzyme and inhibit ATP hydrolysis and ion transport. (c) Binding of palytoxin to Na⁺/K⁺-ATPase. Palytoxin (PTX) binds either to the E₁ or to the E₁-P conformation of the enzyme and arrests the natural ionophore of the sodium pump in a permanently open state (step 3), resembling the situation of an activated ion channel. Like the latter, the palytoxin-enzyme complex also allows Na⁺ or K⁺ cations to flow down their concentration gradients. The dotted line around the phosphate indicates that phosphorylation is not absolutely required.

named *plasmERosome* (Fig. 3) [4]. This may explain why a partial inhibition of the sodium pump isoforms α₂ and α₃ located here results in a small, transient increase in the sub-plasmalemmal Na⁺ concentration, which activates NCX1 and leads to a small local rise of Ca²⁺. This, in turn, stimulates the release of more Ca²⁺ from the ER/SR to the cytoplasm. In arterial smooth muscle cells this pathway is postulated to lead to arteriolar contraction and might be one of the reasons for essential hypertension.

Nevertheless, numerous recent investigations prove that interaction of CTS with the Na⁺/K⁺-ATPase located in caveolae at the plasma membrane leads to a series of events that trigger various intracellular signaling cascades. The same results are also obtained when CTS interact with nonpumping sodium pump mutants [5], indicating that a local [Na⁺] elevation followed by a [Ca²⁺]_i rise is not necessarily required for the induction of the signaling process. In addition, CTS-induced signaling events might differ depending upon the cell

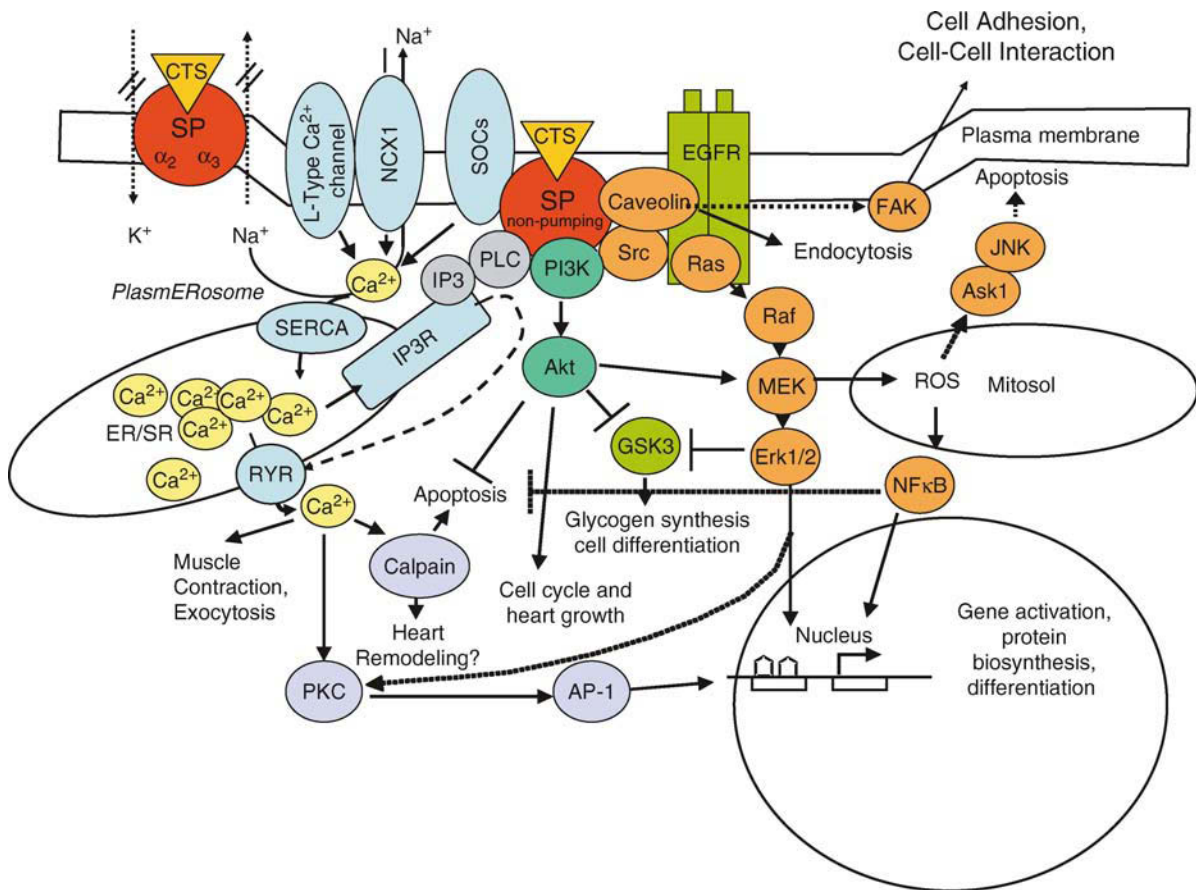
type and the nature of the CTS used. Figure 3 summarizes a series of signaling cascades proposed to explain the results from numerous investigations in various tissues. Thus, binding of CTS leads to a direct interaction of the amino-terminal end of the catalytic α subunit of Na⁺/K⁺-ATPase with the inositol trisphosphate (IP₃) receptor protein of the ER/SR and/or with phospholipase C (which raises IP₃ levels) leads subsequently to a rise of [Ca²⁺]_i. This promotes a positive inotropy of the heart, arterial smooth muscle contraction, exocytosis, and leads to **remodeling of the heart**. Additionally, such effects may be induced by direct interaction of Na⁺/K⁺-ATPase within the plasma membrane with L-type Ca²⁺ channels. Elevated [Ca²⁺]_i leads to an activation of protein kinase C (PKC), which activates cardiac genes via **transcription factor AP-1**. This may lead to cardiac hyperplasia. Besides these possibilities, CTS may activate via the Na⁺/K⁺-ATPase-Src-caveolin-epidermal growth factor complex the Ras-Raf-MEK-ERK1/2 pathway. CTS-induced



Na^+/K^+ -ATPase. Figure 2 Specific Inhibitors of Na^+/K^+ -ATPase. (a) Endogenous cardiac glycosides identified in mammals. Substances with a 5-membered lactone at position C17 of the steroid moiety are referred to as cardenolides, those with a 6-membered lactone as bufadienolides. (b) Palytoxin ($\text{C}_{129}\text{H}_{223}\text{N}_3\text{O}_{54}$) produced by corals of the genus *Palythoa*.

activation of the Ras-Raf-MEK-ERK1/2 pathway may also activate the formation of reactive oxygen species (ROS) in mitochondria that stimulate nuclear factor κB (NF κB). In kidney tubule and heart cells, activation of

genes by this route leads to differentiation of the tissue. In tumor cells, cardiac glycosides activate apoptosis via Ask1 and JNK, leading thereby to cell death, whereas in heart and kidney cells stimulation of ROS formation



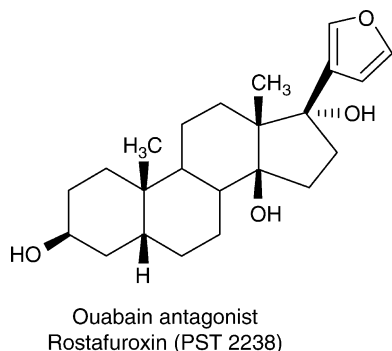
Na⁺/K⁺-ATPase. Figure 3 Mechanism of action of exogenous and endogenous cardiac glycosides via the sodium pump in the plasmERosome and in the signalosome. The Na⁺ lag hypothesis assumes the existence of a sub-plasmalemmal space called the PlasmERosome. A cardioactive steroid (CTS)-dependent transient local rise of [Na⁺]_i in this compartment caused by stimulation of store-operated channels (SOC) or by CTS-dependent inhibition of α₂ and α₃ sodium pump subunits may subsequently lead to a local rise of [Ca²⁺]_i. This signal is amplified by filling up the sarco/endoplasmic reticulum (SR/ER) via the sarco/endoplasmic Ca²⁺-ATPase (SERCA) to a higher extent with Ca²⁺. Any agonist-stimulated Ca²⁺ release leads to an amplified Ca²⁺ release via the inositol tris-phosphate receptor (IP₃R) that stimulates the opening of the ryanodine Ca²⁺ channel (RyR), thus resulting in a higher degree of vasoconstriction. In a non-inhibited Na⁺/K⁺-ATPase Ca²⁺ in the PlasmERosome will be extruded by the Na⁺/Ca²⁺ exchanger (NCX1) and the plasmalemmal Ca²⁺-ATPase. When Na⁺/K⁺-ATPase acts as a signalosome in caveolae, endogenous and exogenous CTS induce various signal transducing pathways, even with a non-pumping enzyme. The abbreviations used are: AP-1 (activator protein 1), Ask-1 (apoptosis signal-regulating kinase 1), ERK 1/2 (extracellular signal-regulated kinases 1 and 2), FAK (focal adhesion kinase), GSK3 (glycogen synthase kinase 3), IP₃ (inositol (1,4,5) trisphosphate) IP₃R (IP₃ receptor), JNK (c-Jun N-terminal kinase), MEK (mitogen activated ERK-activating kinase, a MAP kinase), MKK (MAP kinase kinase), NCX1 (Na⁺/Ca²⁺ exchanger), NF-κB (nuclear factor κB), PI3K (phosphoinositide 3Y-kinase), PKC (protein kinase C), PLC (phospholipase C), Raf (Raf, a MAPK kinase kinase), Ras (a MAP kinase kinase), ROS (reactive oxygen species), SOC (store-operated Ca²⁺ channel), Src (sarcoma kinase), SP (sodium pump).

leads to the activation of NFκB and inhibition of apoptosis. The amino terminal end of the α subunit of Na⁺/K⁺-ATPase also interacts with phosphatidylinositol 3Y-kinase (PI3K). CTS binding to Na⁺/K⁺-ATPase may thus activate protein kinase B (Akt), which induces hypertrophy of the tissue and blocks apoptosis simultaneously. Akt also inactivates glycogen synthase kinase (GSK-3 α/β), which stimulates glycogen

synthesis and is a master switch in regulating cell fate, specificity, and tumorigenesis. Binding of CTS to the Na⁺/K⁺-ATPase-Src-caveolin-epidermal growth factor complex stimulates the focal adhesion kinase (FAK), which alters cell adhesion and cell-cell interactions but also induces the endocytosis of Na⁺/K⁺-ATPase. This may then lead to the degradation of CTS. It is unclear whether the sodium pump is able to be recycled.

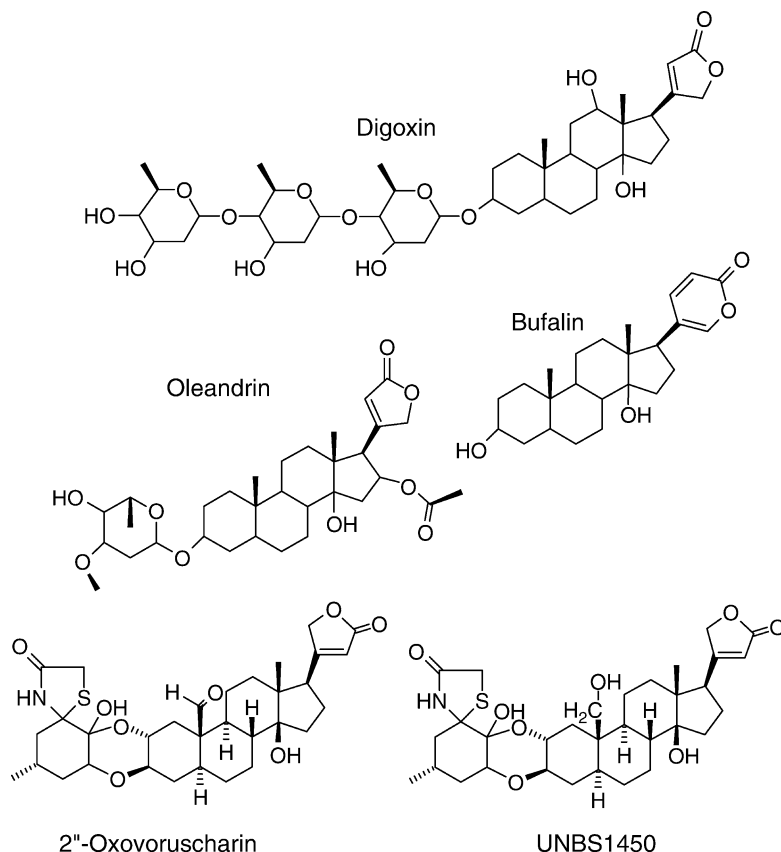
Endogenous Cardiac Glycosides Act as Hormones

A great number of recent investigations have provided evidence for the existence of endogenous cardiac glycosides in animals that act as hormones at nanomolar concentrations [4]. Cardiac glycosides are steroid derivatives with an unsaturated lactone ring at the C17 position. Cardenolides and bufadienolides are generally considered to be of plant origin. However, bufadienolides have been known for a long while to be synthesized by amphibians. Recently, cardiac glycosides were isolated and identified in mammals (Fig. 1). The



Na^+/K^+ -ATPase. **Figure 4** Ouabain antagonist.

cardenolide ouabain was isolated from human blood and bovine adrenal glands and hypothalamus. Digoxin was isolated from urine of humans not being treated with this drug. The bufadienolides marinobufagenin, telocinobufagenin and 19 norbufalin were isolated from human fluids and tissues. There is a high probability that the zona fasciculata cells of the adrenal cortex, and possibly also the hypothalamus, are able to synthesize cardenolides. An adrenal tumor secreting ouabain and producing hypertension has been described. Na^+ -dependent release of ouabain, angiotensin II, endothelin, and catecholamines from cells of the midbrain may stimulate the release of endogenous ouabain from adrenal glands. An increase of sympathetic tone in stress and physical exercise leads to a rapid rise of endogenous ouabain and to a rapid fall after discontinuation. A constant and small rise of ouabain plasma concentrations (in the nanomolar concentration range, either from endogenous or exogenous sources) leads to arterial hypertension and remodeling of the heart and the arterial wall. Fifty percent of Caucasians with uncomplicated arterial essential hypertension and low plasma renin activity have been shown to exhibit elevated concentrations of endogenous ouabain. Possibly, endogenous digoxin, whose biosynthesis has



Na^+/K^+ -ATPase. **Figure 5** Cardiac glycosides and their derivatives with anti-cancer action.

also been demonstrated to take place in the adrenal gland, counteracts the secretion of endogenous ouabain from midbrain cells and lowers the sympathetic tone. This suppressive effect of digoxin could represent a very important mechanism for the therapeutic action of exogenously applied digoxin as well. There is good evidence that the secretion of the bufadienolide marinobufagenin from adrenal glands is activated via angiotensin II and catecholamines from the adrenal glands when the Na⁺ concentration increases in midbrain and hypothalamus. Marinobufagenin and telocinobufagenin are also elevated in essential hypertension. Contrary to the case with other cardenolides, marinobufagenin shows a natriuretic action on kidney tubular cells containing mainly the α_1 isoform of Na⁺/K⁺-ATPase [4].

Rostafuroxin, a Cardenolide Derivative with Antihypertensive Action

If 50% of Europeans with essential hypertension are affected by this disease because of an elevated secretion of endogenous ouabain, then there might be a chance to block its interaction at the cardiac glycoside binding site of Na⁺/K⁺-ATPase and thus lower blood pressure. This therapeutic approach seems to be successful. Recent studies provide evidence that the cardenolide analogue Rostafuroxin (PST 2238; Fig. 4) at very low concentrations can overcome the ouabain-induced rise of hypertension in experimental animals [6]. This compound has recently entered the phase I of clinical trials and is certainly a prototype of a new class of antihypertensive drugs.

Cardiac Glycosides as Anti-Cancer Agents

There is increasing evidence that proliferation of cancer cells is affected by cardiac glycosides and that the extent of this effect depends on the nature of the steroid derivative and on the cancer cell line used. Thus, digitalis or ouabain have been shown to induce apoptosis in human breast cancer or neuroblastoma cells, respectively, while bufalin and the class of the more hydrophobic cardenolides like digoxin, olean-drin, and the 2''-oxovoruscharin and its derivative UNSB1450 (Fig. 5) have been shown in vitro and in vivo to have anti-cancer activity primarily in leukaemia, lymphoma, melanoma, and ovarian and prostate cancer cells. These new investigations point toward new fields of research with the perspective of evaluating a potential introduction of CTS as anti-cancer agents in the treatment of malignancies. UNSB1450 seems especially to have a prospective benefit for cancer therapy. The compound has entered phase I clinical trials in Belgium [7].

- ▶ Na⁺/H⁺ Exchangers
- ▶ Cardiac Glycosides

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Na⁺ K⁺ 2Cl[−] Cotransporter

N

The bumetanide-sensitive Na⁺, K⁺, 2Cl[−] cotransporter (NKCC) mediates the electroneutral uptake of chloride across epithelial cell membranes and is found in both absorptive and secretory epithelia (airways, salivary gland). NKCC exists in two isoforms, the secretory isoform NKCC1, and the absorptive isoform NKCC.

NKCC is a heavily glycosylated protein with 12 putative membrane-spanning regions. Thirty percent of the sodium that is filtered by renal glomeruli is reabsorbed by Na-K-2Cl cotransport in the ascending limb of Henle in the nephron. Na-K-2Cl cotransport is a target of all loop diuretics.

- ▶ Antihypertensive Drugs
- ▶ Diuretics

Na⁺-Proton Exchanger

- ▶ Na⁺/H⁺ Exchangers

NADPH-diaphorase

NADPH-diaphorase activity is the ability of an enzyme to reduce soluble tetrazolium salts to an insoluble, visible formazan. This activity is being used by many laboratories to localize NO synthase histochemically.

► NO Synthases

NANC Transmission/Mediators

► Non-adrenergic Non-cholinergic (NANC) Transmission/Mediators

Narcolepsy

Narcolepsy is a chronic neurological disorder caused by the brain's inability to regulate sleep–wake cycles normally. At various times throughout the day, people with narcolepsy experience fleeting urges to sleep. If the urge becomes overwhelming, patients fall asleep for periods lasting from a few seconds to several minutes. The daytime sleep attacks are often accompanied by catalepsy, a sudden loss of muscular tone as usually observed during REM sleep, causing the patient to physically collapse. In a similar way, during the night, the patients have difficulty in maintaining *sleep continuity*. Thus, narcolepsy describes a disorder in which the affected individuals experience frequent and unpredictable transitions between wakefulness and sleep. The disorder has been linked to dysfunction in the *orexin* system, which in healthy individuals is (among other roles) responsible for stabilising the sleep/wake states.

► Sleep ► Orexins

Narcotics

► General Anaesthetics

Natriuretic Drugs

► Diuretics

Natriuretic Peptides

Natriuretic peptides are a family of peptide hormones. All of them contain a 17-amino acid long ring that is closed by a disulfide bond between two cysteine residues. ANP (atrial natriuretic peptide) is mainly expressed in the atria of the heart, whereas BNP (B-type natriuretic peptide) is synthesized in the ventricular myocardium. CNP occurs mainly in the endothelium and is thought to have a paracrine function. ANF and BNF lower blood pressure by a direct effect on smooth muscle and on the salt retention in the kidney. Natriuretic peptides bind and activate particulate guanylyl cyclases.

► Guanylyl Cyclase

Natural Killer Cells

Large granular lymphocytes, not belonging to either the T- or B-cell lineage. Natural killer (NK) cells are considered part of the innate defense system since, in contrast to cytotoxic T-cells, they are able to kill certain tumor cells in vitro without prior sensitization. The basal activity of NK cells increases dramatically following stimulation with type I IFNs. In addition, NK cells display Fc-receptors for IgG and are important mediators of Antibody-Dependent-Cell-mediated-Cytotoxicity (ADCC).

► Immune Defence

Nausea

► Emesis

NCX

► $\text{Na}^+/\text{Ca}^{2+}$ Exchangers

NDP-dependent K^+ Channel

The NDP-dependent potassium (K_{NDP}) channel is activated by nucleoside diphosphates (NDPs) in the presence of Mg^{2+} and is relatively insensitive to inhibition by ATP. K_{NDP} channels are present in vascular smooth muscle.

► ATP-dependent K^+ Channels

Necrosis

Necrosis is a form of cell death, which involves multiple cells at the same time, all of which share a similar susceptibility. For example, blockage of an artery to an organ leads to anoxia to all of the cells of the tissue and leads to the death of these cells, which is called necrosis. Necrosis can also be seen in response to pharmacologic agents at very high doses, such as suicidal doses of acetaminophen. The process of cell death by necrosis can share some mechanisms with that of single cell death or apoptosis. For example, when similar cytotoxic drugs are given at much lower doses, single cells die by apoptosis rather than by necrosis. Another example is when the cell death process leads to depletion of ATP as it does with apoptosis and then extends to a secondary aggravation of the mitochondrial lesions leading to more extensive ATP depletion, which causes multiple cells to die synchronously; this is called necrosis.

► Apoptosis

NEP

► Neutral Endopeptidase

Nephrogenic Diabetes Insipidus

In nephrogenic diabetes insipidus the kidney's ability to respond to AVP is impaired by different causes, such as drugs (e.g. lithium), chronic disorders (e.g. sickle cell disease, kidney failure) or inherited genetic disorders (X-linked or autosomal NDI). This type of diabetes insipidus can not be treated by exogenous administration of AVP or AVP analogues. Instead, diuretics (hydrochlorothiazide combined or not with amiloride) and NSAIs (indomethacin) are administered to ameliorate polyuria.

► Vasopressin/Oxytocin

Neprilysin

► Neutral Endopeptidase

Nerve Growth Factor

► NGF

► Neurotrophic Factors

Neuraminidase Inhibitors

Neuraminidase inhibitors are the major class of drugs to treat or to prevent the infection with influenza viruses. Currently, two neuraminidase inhibitors are available, zanamivir and oseltamivir, which block the release of new influenza virus from infected host cells and thereby stop the spread of infection. The enzyme neuraminidase is a surface glycoprotein present on all influenza viruses. There are nine influenza neuraminidase subtypes known of which subtypes N1 and N2 appear to be the most important ones. Neuraminidase inhibitors are effective against all neuraminidase subtypes. The activity of the neuraminidase is required for the newly

formed virus to detach from the host cell. To achieve that, neuraminidase cleaves terminal sialic acid residues from carbohydrate moieties on surface proteins of the host cell to which the newly formed virus particles are attached. This cleavage then results in the release of the viruses which are now able to infect other cells. Neuraminidase inhibitors are generally well tolerated. While zanamivir has to be administered by inhalation, oseltamivir can be given orally.

► Antiviral Drugs

Neuraxis

The neuraxis is the rostrocaudal extension of the nervous system including forebrain, midbrain, brainstem, spinal cord, and peripheral nerves.

► Analgesics

Neuregulin

Neuregulins are a complex family of factors consisting of neuregulins-1, -2, -3 and -4, that perform many functions during morphogenesis of various organs. Neuregulins exert their effects by activating the ErbB-family of receptor tyrosine kinases. While neuregulin-1 and -2 bind both ErbB3 and ErbB4, neuregulin-3 and -4 bind only ErbB4. Neuregulin-1 is also known as “neuro differentiation factor” or “heregulin”.

Neurodegeneration

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Synonyms

Neuronal cell deterioration; Neuronal cell death

Definition

Neurodegeneration refers to the processes whereby damaged neuronal cells deteriorate or degenerate and eventually die.

Since the body's ability to replace lost neurons (i.e., such as via neurogenesis) is quite limited when compared to many nonneuronal cells, degenerative processes affecting neurons can be quite devastating.

Basic Mechanisms

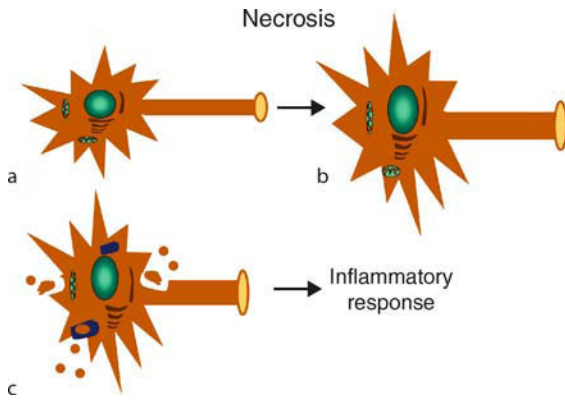
The basic mechanisms underlying the neuronal cell degeneration and death observed in the neurologic disorders as diverse as Alzheimer's disease and stroke have not been fully elucidated. However, a number of distinct factors and processes clearly contribute to neurodegeneration including increased oxidative stress and free radical damage, impaired mitochondrial function, excitotoxicity, immunologic and inflammatory mechanisms, impaired trophic factor support, and altered cell signaling. In the process of neuronal degeneration, cells eventually die as a result of one of two processes, apoptosis or necrosis.

Necrosis

Necrosis (sometimes referred to as cellular dissolution) occurs as a pathological response to cell injury most commonly resulting from trauma, ischemia, hypoxia, neurotoxins, or infection. Necrosis occurs when a cell is too severely damaged for the orderly energy-dependent process of apoptosis (see below) to occur. Following one or more of the insults listed above, neuronal degeneration or death occurs in groups of contiguous cells in a localized region and the initiation of inflammatory processes can be clearly observed in tissue sections [1]. In the process of necrosis, an initial swelling of the cell occurs (Fig. 1 and Table 1), little or no chromatin condensation is evident, mitochondria and other organelles swell and rupture, the plasma membrane lyses, and spillage of the cellular contents into the extracellular space follows. A general inflammatory response is then triggered and macrophages attack and phagocytize the cellular debris.

Apoptosis

Neuronal apoptosis (old Greek term for dropping off, like leaves in the fall) is triggered by a number of factors including lipid peroxidation (and membrane damage) induced by reactive oxygen species, genetic mutation, or DNA damage (or degradation) resulting from radiation or other destructive agents. A loss of trophic factor support, as well as some of the same factors that induce necrosis (see above) can also initiate apoptotic processes. Cell death by apoptosis also occurs extensively during the development of the mammalian nervous system and is required for the formation of appropriate connections between neurons and their



Neurodegeneration. Figure 1 Illustration of the major cellular changes observed in neuronal necrosis. A normal neuronal cell (a) when exposed to an insult (e.g., trauma, ischemia, hypoxia, neurotoxins, infection, etc.) initially swells (b), mitochondria and other organelles swell and rupture, the plasma membrane lyses (c), and spillage of the cellular contents into the extracellular space follows. A general inflammatory response (d) is then triggered and macrophages attack and phagocytize the cellular debris.

Neurodegeneration. Table 1 Comparison of necrosis and apoptosis

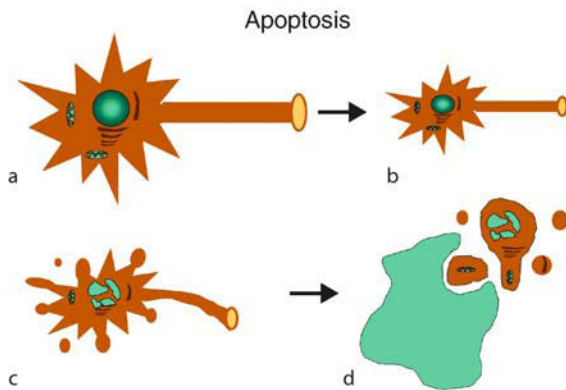
Necrosis	Apoptosis
Cellular swelling	Cellular shrinkage
	Nuclear and cellular pyknosis
Little or no chromatin condensation	Chromatin condensation
Rupture of organelles and plasma membrane	Organelles and plasma membrane not usually ruptured
Release of cytoplasmic contents and inflammation	Release of cytoplasmic contents and inflammation not usually present
Random DNA degradation	DNA fragmentation
Caspases not involved	Activation of caspases
	Cytoplasmic blebbing
	Formation of apoptotic bodies which are engulfed and cleared by phagocytes

targets. The processes involved in apoptosis differ from necrosis (Table 1) in several important details, most notably, that neuronal death with apoptosis usually involves individual cells that are phagocytized before they can release their cytoplasmic contents and induce an inflammatory response in adjacent tissues, and

phagocytes are able to recognize dying or degenerating cells by their expression of death related cell surface epitopes. Furthermore, mitochondria are preserved until the late stages of apoptosis, whereas they swell and disintegrate early in necrosis. Figure 2 illustrates the major cellular changes observed in neuronal apoptosis. A normal neuronal cell (a) when exposed to specific triggers (e.g., lipid peroxidation, genetic mutation, DNA damage, excitotoxic injury, etc.) initially shrinks, chromatin becomes pyknotic (more dense) and condenses, then migrates to the nuclear membrane, DNA fragmentation and degradation occurs, and several organelles disappear (e.g., Golgi apparatus, endoplasmic reticulum). Afterwards, blebbing of the plasma membrane occurs (c), the cell then fragments into small apoptotic bodies that are subsequently phagocytosed and digested (d) without triggering inflammation.

As mentioned above, neuronal apoptosis serves a number of important roles in normal brain development and is a key mechanism by which defective or damaged neurons are removed from the brain. However, in a number of brain disorders including Alzheimer's disease, Dementia with Lewy bodies, and Parkinson's disease, inappropriate apoptosis may occur leading to accelerated neuronal loss and progressive disease symptoms. On the other hand in neural tumor cells, such as neuroblastoma and medulloblastoma cells, apoptotic pathways may be disabled and the cells become resistant to chemotherapeutic drugs that kill cancer cells by inducing apoptosis. Neuroblastoma and medulloblastoma are important pediatric solid tumors that arise in the sympathetic neuron lineage and cerebellum, respectively. Apoptosis may be accelerated or retarded by a variety of hormones, metabolic byproducts, electrolytes, and other endogenous substances. For example, altered serum levels of thyroid hormone or ammonia, altered plasma or extracellular levels of excitatory amino acids such as glutamate and aspartate, imbalances of calcium and other electrolytes, and lactic acidosis are all known to initiate or modify apoptotic processes. Apoptosis is also influenced by synaptic communication in both the central and peripheral nervous systems. For example, in transsynaptic degeneration, neurons deteriorate and often undergo apoptosis if they fail to be innervated (from the afferent side) due to the loss of presynaptic neurons. This process has been observed in the lateral geniculate body after optic nerve lesions and in the inferior olivary nucleus after destruction of the central tegmental tract. Efferent, motor neurons degenerate if they fail to match with target muscle fibers or their muscle targets are lost, such as after amputation of a limb.

There is an increasing body of evidence that supports an apoptosis–necrosis cell death continuum. In this continuum, neuronal death can result from varying contributions of coexisting apoptotic and necrotic mechanisms [2]. Therefore the distinct designations



Neurodegeneration. Figure 2 Illustration of the major cellular changes observed in neuronal apoptosis. A normal neuronal cell (a) when exposed to specific triggers (e.g., lipid peroxidation, genetic mutation, DNA damage, excitotoxic injury, etc.) initially shrinks, chromatin becomes pyknotic and condenses, then migrates to the nuclear membrane, DNA fragmentation and degradation occurs, and several organelles disappear. Afterwards, blebbing of the plasma membrane occurs (c), the cell then fragments into small apoptotic bodies that are subsequently phagocytosed and digested (d) without triggering inflammation.

above (necrosis vs. apoptosis) are beginning to blur. [Table 1](#) provides an overview of the major differences between necrosis and apoptosis.

Regulation of Apoptosis

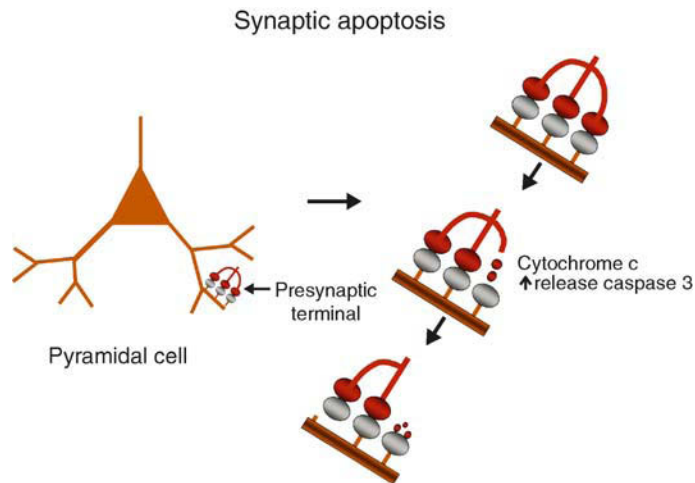
Apoptosis is regulated by a complex molecular cascade that controls the activation of a family of cysteine proteases known as caspase proteins (caspases 1–14). Caspases are responsible for breaking down vital structural and functional proteins, leading to the characteristic cytomorphology associated with apoptosis. While multiple molecular pathways (e.g., mitochondrial, death receptor, endoplasmic reticulum pathways) have been identified that lead to the activation of caspases, the mitochondrial (intrinsic) pathway is most associated with neuronal apoptosis. The mitochondrial pathway regulates caspase activity by controlling mitochondrial release of cytochrome *c* by pro- and antiapoptotic members of the Bcl-2 family of proteins. These proteins interact through dimerization in the mitochondrial membrane. The ratio of proapoptotic (e.g., Bax, Bad) to antiapoptotic (e.g., Bcl-2, Bcl-XL) protein levels is a key determinant in regulating cytochrome *c* release and subsequent caspase activation. For example, high Bax/Bcl-2 and Bax/Bcl-XL ratios promote cytochrome *c* release while low Bax/Bcl-2 and Bax/Bcl-XL ratios inhibit cytochrome *c* release. Downstream of mitochondria, cytochrome *c* forms a complex with caspase-9 and Apaf-1 to form the apoptosome. This complex cleaves procaspase-3 to form activated

caspase-3, which can initiate cellular disassembly. While caspase-3 is known as a downstream effector caspase, its activity can still be inhibited by members of the inhibitor-of-apoptosis (IAP) protein family (e.g., XIAP); XIAP is itself potentially inhibited by Smac/Diablo, a mitochondrial protein (reviewed [5] [3]).

As noted above, apoptotic activity can be triggered by a broad array of stimuli including oxidative stress (e.g., ischemia, hypoxia), proinflammatory cytokines, excitotoxicity, neurotrophin withdrawal, mitochondrial dysfunction, and abnormal intracellular calcium concentrations. A number of these stimuli can alter Bcl-2 family protein expression via potent regulatory genes such as *p53* and *par-4* in order to promote cytochrome *c* release and induce caspase-3 activation. Increased neuronal apoptosis has been demonstrated in classic neurodegenerative disorders including Alzheimer's disease. Interestingly, these disorders are also characterized by alterations in apoptotic regulatory proteins including several Bcl-2 family proteins and caspases. For example, Bcl-2 and caspase-3 are increased in Alzheimer's cortex. These increases are thought to be due to a compensatory upregulation in response to the neurodegenerative process. In contrast, lower Bcl-2 levels have been reported in frontal cortex in patients with autism, a classic neurodevelopmental disorder. Accumulating data suggest important roles for apoptotic pathways in the pathophysiology of a spectrum of neuropathological disorders (reviewed [5] [3]).

Localized Apoptotic Activity in Synapses and Neurites

Although caspase activation is often considered a precursor to rapid cell death, the emerging concept of synaptic or neuritic apoptosis suggests that apoptotic activation can be localized to synapses or distal neurites without inducing immediate neuronal death or involving the neuronal cell body ([Fig. 3](#)). Localized apoptotic mechanisms may lead to the release of cytochrome *c* from the mitochondria and an increase in the concentration of activated caspase-3 in a presynaptic terminal that is synapsing on a dendritic spine. Increased caspase-3 activity results in a localized breakdown of this nerve terminal and its synapse. Subsequently, the postsynaptic dendritic spine retracts and disappears. This process could also begin in the postsynaptic dendritic spine. Although mitochondria are usually not found in dendritic spines, dendritic mitochondria may release cytochrome *c* which can then diffuse into the dendritic spine. A dendritic spine-localized increase in caspase-3 activity could subsequently eliminate the spine. In Alzheimer's disease, this form of synaptic loss is thought to contribute to the initial cognitive decline that predates the onset of large-scale neuronal death. In the peripheral and central nervous systems, developmental and injury-induced synapse elimination has



Neurodegeneration. Figure 3 Illustration of synaptic (neuritic) apoptosis. A pyramidal neuron is depicted with cortical afferents synapsing on its dendrites. Localized apoptotic mechanisms lead to the release of cytochrome c from the mitochondria and an increase in the concentration of activated caspase-3 in a presynaptic terminal that is synapsing on a dendritic spine. Increased caspase-3 activity results in a localized breakdown of this nerve terminal and its synapse. Subsequently, the postsynaptic dendritic spine retracts and disappears (Figure modified from Glantz et al. [5] [3]).

been hypothesized to occur via axon retraction after the postsynaptic cell either withholds trophic support or actively promotes synaptic breakdown. Interestingly, caspase-3 activity has also been associated with normal physiological activity, including synaptic plasticity. For example, caspase-3 is involved with axonal regeneration associated with retinal growth cone formation, demonstrating roles in both local protein synthesis and degradation. Caspase activity has also been associated with long-term spatial memory storage (associated with behavioral training) in the rodent hippocampus and has been localized to dendrites, dendritic spines, and axon terminals in the rat forebrain. Collectively, these studies demonstrate that apoptosis localized to synapses and distal dendrites represents a potential mechanism underlying synaptic remodeling and elimination in both physiological and pathological conditions (reviewed [5] [3]).

Important Mediators of Neurodegeneration

Oxidative Stress

A variety of metabolic pathways generate highly reactive by-products known as free radicals including hydrogen peroxide, superoxide anions, and hydroxyl radicals. These substances can be used by various cells as part of the immune response to serve useful functions such as to combat infectious organisms and neoplastic cells, and to execute cells programmed for death during the normal course of development. In abnormal circumstances such as associated with traumatic and ischemic injury or neurodegenerative diseases such as Alzheimer's and Parkinson's disease, free radicals may be excessively

produced, aberrantly controlled, or inadequately scavenged. In such cases, free radicals cause injury as a result of membrane lipid peroxidation, DNA damage, iron accumulation, and protein nitrosylation. Excess free radicals are normally scavenged and inactivated by several endogenous substances such as vitamin E (α -tocopherol) which can quench lipid peroxidation, superoxide dismutase which scavenges superoxide radicals, and glutathione peroxidase which removes hydrogen peroxide and lipid peroxides. Therefore alterations or deficits in any of these endogenous substances can contribute to and/or initiate neurodegeneration.

Excitotoxic Amino Acids

Excitotoxic amino acids play a deleterious role in a number of neurologic diseases and are known to contribute to neurodegeneration. These compounds are released in response to a wide variety of insults to the CNS and include glutamate, aspartate, and several oxidation products of cysteine and homocysteine. For example, in stroke, excitatory amino acids are released in the penumbra of ischemic lesions and further released when perfusion is restored, and thus are believed to contribute significantly to reperfusion injury. These compounds are also released following traumatic brain injury, during prolonged seizures, and are thought to contribute to the neurotoxicity associated with the amyloid plaques observed in Alzheimer's disease. Over-activation of N-methyl-D-aspartate (NMDA) receptors (a subtype of glutamate receptor) by glutamate leads to alterations in a number of signal systems and ion channels activating apoptosis.

Energy Failure and Ion Dysregulation

Neuronal degeneration may result as a consequence of energy failure within mitochondria precipitated by ischemia, free radical damage, and several acquired and genetic disorders of metabolism. For example, mitochondrial energy disruption and neurodegeneration occur in Wernicke's encephalopathy, an acquired metabolic disorder resulting from ethanol abuse and/or thiamine deficiency. Similar neuropathology can be observed in Leigh's syndrome, an inherited neurometabolic disorder in which point mutations in mitochondrial DNA are evident. Friedrich's ataxia, the most common inherited ataxia, is an autosomal recessive disease in which protein aggregates appear to disrupt mitochondrial iron metabolism, leading to abnormal free radical formation and altered energy metabolism. In the cases highlighted above, specific irreversible processes lead to a decrease in high energy phosphates (e.g., ATP, creatine phosphate), possibly leading further to elevated acyl-CoA levels that inhibit multiple metabolic processes. Local electrolyte (ion) imbalances and/or ion channel dysfunction are also thought to contribute significantly to neurodegenerative processes. Ion changes are commonly among the early events in apoptosis and fact, alterations in calcium homeostasis are among the best-documented factors in neurodegeneration. Direct evidence of the importance of ion channels in neurodegeneration comes from genetic disorders that affect specific ion channels (i.e., channelopathies). Channelopathies may underlie certain forms of migraine, episodic ataxias, and epilepsy. Indirect evidence that ion dysregulation plays an important role in some forms of neurodegeneration comes from preclinical studies (i.e., stroke models in animals) in which calcium and sodium channel blockers reduce infarct size.

Inflammation

Several lines of evidence indicate that inflammatory processes contribute to the neurodegeneration found in a number of disease states. A common feature in neurodegenerative diseases is microgliosis. Microglia, in addition to releasing oxygen free radicals, also secrete a variety of compounds and substances known to stimulate local inflammation such as inflammatory cytokines, complement and coagulation proteins, as well as binding proteins. As an example, inflammatory factors found in degenerating sites in Alzheimer's disease brains include activated microglia, the cytokines interleukin Il-1 and Il-6, an early component of the complement cascade, C1q, as well as acute phase reactants such as C-reactive protein.

Neurotrophin Support and Altered Cell Signaling

A continuous supply of a variety of polypeptide molecules known as neurotrophic factors (or neurotrophins) is essential to the nervous systems of all vertebrates throughout development as well as in adult

life [4]. These important molecules interact with specific receptors and initiate a variety of cellular signaling systems. During the period of target innervation, limiting amounts of neurotrophic factors regulate neuronal numbers by allowing survival of only some of the innervating neurons, the remaining being eliminated by apoptosis. Increasing evidence indicates that several neurotrophic factors also influence the proliferation, survival, and differentiation of precursors of a number of neuronal lineages. In the adult, neurons continue to be dependent on trophic factor support, which may be provided by the target or by the neurons themselves. Altered trophic factor support and cell signaling as a result of excess free radicals or peroxynitrites has been implicated in the neurodegenerative processes associated with several neurologic diseases. Furthermore, the ability of neurotrophins to promote survival of peripheral and central neurons during development and after neuronal damage has stimulated the interest in these molecules as potential therapeutic agents for the treatment of nerve injuries and neurodegenerative diseases. Examples of important (therapeutically relevant, from a neurodegenerative disease standpoint) neurotrophins include nerve growth factor (NGF), and brain derived growth factor (BDNF) (see further discussion below).

Pharmacological Intervention

There are multiple mechanisms known to underlie the neuronal cell damage associated with injury or disease that at least theoretically could be targeted for pharmaceutical intervention. Currently however, there is no clinically available therapeutic agent that can reliably protect the brain from progressive neurodegenerative processes for sustained periods. Due to the extensive amount of preclinical research that has been conducted in recent years, there is a basis for optimism, however, it appears likely that some of these approaches will result in clinically effective therapeutic modalities in the near future. A short overview of some of the investigational approaches to combat neurodegeneration appears below.

Inhibitors of Inflammatory Processes

Inflammatory processes associated with neurodegenerative disease suggest a number of therapeutic targets, including inhibitors of complement activation or cytokines, free radical scavengers, and inhibitors of microglial activation. In retrospective studies, the use of nonsteroidal antiinflammatory drugs (NSAIDs) has been associated with a reduced incidence or slowed progression of Alzheimer disease, indicating a potential for therapeutic use of this class of agent.

Inhibitors of Apoptosis and Growth Factor like Molecules

As indicated earlier, apoptosis is inhibited by certain proteins, such as Bcl-2 and Bcl-x. In contrast, Bax and

the tumor suppressor protein, p53, have been shown to enhance the onset of apoptosis. Accordingly, drugs which have the ability to enhance the expression of Bcl-2 and Bcl-x or to inhibit the expression Bax and p53 could theoretically have the potential to reduce neurodegeneration [5]. Drugs that inhibit apoptosis-inducing enzymes including caspases may also have a role. The expression of Bcl-2 and other proteins in this family is also modulated by trophic factors such as NGF and basic fibroblast growth factor (FGF), endogenous neurotrophins which have been shown to block cell death and preserve the phenotype of various cells in the nervous system. NGF is well known to support basal forebrain cholinergic neurons, cells reproducibly ravaged in Alzheimer's disease and known to be critically important for many cognitive processes. Accordingly, there has been interest in using NGF as a therapeutic modality for Alzheimer's disease and potentially other conditions in which cholinergic deficits may be present (e.g., dementia with Lewy bodies). In other investigations, the potential role of trophic molecules in stroke has been evaluated. For example, in animal models of stroke, ischemic damage is reduced following treatment with FGF. Unfortunately, NGF, FGF, as well as most other peptide molecules fail to adequately penetrate the brain from peripheral administration and are thus considerably limited from a therapeutic standpoint. Recent interest has thus focused on low molecular weight growth factor like molecules or small organic molecules that increase the release of growth factors in the brain or increase the expression of growth factor receptors.

Inhibitors of Oxidative Stress

Human trials have evaluated vitamin E, selegiline, and other antioxidant molecules for their ability to prevent or slow the progressive neurodegeneration associated with several neurologic diseases. To date, the data have provided conflicting or equivocal results with some studies showing slightly positive effects and others showing little or no effect. A number of issues require further attention in this area such as the identification of optimal doses of the various antioxidant compounds as well as the evaluation of selected combinations of these agents. These issues are important since specific compounds are known to scavenge or inactivate specific oxidative agents, and thus a single compound would not intuitively be expected to combat free radicals originating from several sources.

Modulators of Glutamate Transmission

Several glutamate antagonists have been or are in the process of being evaluated both preclinically and clinically as neuroprotective agents. For example, MK-801, an NMDA antagonist, reduces the detrimental effects of excess glutamate (as well as other insults to neurons) in a variety of animal models. Unfortunately the

compound is too toxic for use in humans. However, memantine (another NMDA antagonist) is available adjunctively (to be administered with acetylcholinesterase inhibitors) for the treatment of moderate to severe Alzheimer's disease. Riluzole (a drug which inhibits glutamate release), is available clinically for the treatment of amyotrophic lateral sclerosis (ALS) and is somewhat effective in slowing progression. Riluzole has also been shown to reduce infarct size in stroke and brain injury after trauma in animal models and accordingly human studies are anticipated in the near future. Other agents that modulate glutamate receptors such as AMPA antagonists and compounds that interact allosterically at the polyamine and glycine receptor sites are also being evaluated.

Other Investigational Approaches

Other pharmaceutical and molecular therapies are currently being developed to antagonize neurodegenerative processes. For example, compounds designed to prevent toxic reactions of free radicals such as nitric oxide (NO) and reactive oxygen species (ROS), new calcium channel antagonists, as well as compounds that stimulate the expression of antioxidant enzymes such as superoxide dismutase are being developed. Further, the low incidence of cardiovascular disease in those who consume large amounts of omega-3 fatty acids and their known ability to protect cell membranes from a variety of insults has provided the impetus to evaluate these agents as potential neuroprotectants.

In summary, the steadily increasing size of geriatric populations in developed countries and the resultant increases in age-related diseases of the brain have provided the impetus for intensive study of the processes underlying neurodegeneration. A better understanding of these processes will likely lead to better methods of treatment not only for progressive memory disorders such as Alzheimer disease, but also for motor disorders such as amyotrophic lateral sclerosis, and cerebrovascular disorders such as stroke.

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Neurokinin A

- ▶ Substance K
- ▶ Tachykinins
- ▶ Neuroleptics

Neuroleptic-like Malignant Syndrome

Neuroleptic-like malignant syndrome is a serious but very rare adverse effect of some drugs, of e.g., neuroleptics, some anaesthetics and apparently tolcapone. Symptoms include hyperthermia, muscle deterioration, even dissolution.

- ▶ Neuroleptics

Neuroleptics

Neuroleptics or antipsychotics suppress the “positive symptoms” of schizophrenia such as combativeness, hallucinations and formal thought disorder. Some also alleviate the “negative symptoms” such as affective blunting, withdrawal and seclusiveness. Neuroleptics also produce a state of apathy and emotional indifference. Most neuroleptics block dopamine D₂-receptors but some, like clozapine, also block dopamine D₄-receptors or serotonin 5-hydroxytryptamine_{2A}-receptors.

- ▶ Antipsychotic Drugs
- ▶ Dopamine System
- ▶ Synaptic Transmission

Neuromedin B

- ▶ Bombesin-like Peptides

Neuromedin U

Neuromedin U

Neuromedin U is a neuropeptide which is widely distributed in the gut and central nervous system. Peripheral activities of neuromedin U include stimulation of smooth muscle, increase in blood pressure, alteration of ion transport in the gut, control of local blood flow and regulation of adrenocortical function. The actions of neuromedin U are mediated by G-protein coupled receptors (NMU1, NMU2) which are coupled to G_q/11.

Neuromodulators

Mediators acting mostly at the pre-synaptic or pre-junctional level to control the release of neurotransmitters.

Neuromuscular Blocking Agents

- ▶ Muscle Relaxants

Neuromuscular Junction

Neuromuscular junction (NMJ) is the synapse or junction of the axon terminal of motoneurons with the highly excitable region of the muscle fibre's plasma membrane. Neuronal signals pass through the NMJ via the neurotransmitter ACh. Consequent initiation of action potentials across the muscle's cell surface ultimately causes the muscle contraction.

- ▶ Cholinesterases
- ▶ Nicotinic Acetylcholine Receptor

A six aminoacid neurotensin-like peptides synthesized in the same propeptide precursor as neurotensin.

- ▶ Neurotensin/Neuromedin N

Neuronal Cell Death

- ▶ Neurodegeneration

Neuronal Cell Deterioration

► Neurodegeneration

Neuronal Nitric Oxide Synthase

Synonyms

nNOS

Definition

Cytosolic enzyme constitutively present mainly in nervous tissues and activated in the presence of Ca^{2+} and calmodulin.

► Nitric Oxide
► NO-Synthase

Neuronal Secretion

► Exocytosis

Neuropathic Pain

Neuropathic pain is initiated or caused by a primary lesion in the peripheral or central nervous system. The causative agent may be trauma, nerve-invading cancer, herpes zoster, HIV, stroke, diabetes, alcohol or other toxic substances. Neuropathic pain is refractory to most analgesic drugs. Altered sodium channel activity is characteristics of neuropathic pain states.

► Analgesics
► Local Anaesthetics
► Voltage-dependent Na^+ Channels
► Pain and Nociception

Neuropeptide FF

The octa-peptide neuropeptide FF is generated together with a related octa-peptide (neuropeptide AF) from a

common precursor protein. It is involved in nociception and in the modulation of opiate-induced analgesia, morphine intolerance and morphine abstinence. The effects of neuropeptide FF are mediated by G-protein coupled receptors (NPFF1, NPFF2).

Neuropeptide Y

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Synonyms

NPY

Definition

Neuropeptide Y (NPY) is a 36 amino acid polypeptide with tyrosine residues at both ends of the molecule. It is characterised structurally by a “PP-fold” consisting of an extended polyproline helix and an α -helix connected by a β -turn [1]. Based on structural and evolutionary criteria, NPY is closely related to ►peptide YY (PYY) and ►pancreatic polypeptide (PP).

Basic Characteristics

NPY is primarily (but not exclusively) synthesised and released by neurons, which in the peripheral nervous system are predominantly sympathetic neurons [1]. In most cases, NPY acts as a co-transmitter that is preferentially released upon high frequency nerve stimulation. NPY can be metabolised by the enzyme ►dipeptidylpeptidase IV (also known as ►CD26) to generate the biologically active fragment ►NPY_{3–36}.

As to be expected from a peptide that has been highly conserved during evolution, NPY has many effects, e.g. in the central and peripheral nervous system, in the cardiovascular, metabolic and reproductive system. Central effects include a potent stimulation of food intake and ►appetite control [2], anxiolytic effects, anti-seizure activity and various forms of neuroendocrine modulation. In the central and peripheral nervous system NPY receptors (mostly Y_2 subtype) mediate prejunctional inhibition of neurotransmitter release. In the periphery NPY is a potent direct vasoconstrictor, and it potentiates vasoconstriction by other agents (mostly via Y_1 receptors); despite reductions of renal blood flow, NPY enhances diuresis and natriuresis. NPY can inhibit pancreatic insulin release and inhibit lipolysis in adipocytes. It also can regulate gut motility and gastrointestinal and renal epithelial secretion.

In some cell types, e.g. in vascular smooth muscle cells, NPY appears to enhance cell growth.

NPY, PYY and PP act upon the same family of receptors, which are classified together as NPY receptors. Based on IUPHAR recommendations, the NPY receptors are designated by a capital Y and the various receptors within the family are designated by subscript numbers [3]. Five mammalian subtypes of NPY receptors have been cloned and are designated Y₁, Y₂, Y₄, Y₅ and y₆. Among the cloned receptors the Y₁, Y₂, Y₄ and Y₅ receptors represent fully defined subtypes, but no functional correlate of the cloned y₆ receptor has been established to date. The Y₄ receptor preferentially binds PP, whereas NPY (and PYY) are much less potent at the Y₄ receptor than at the other subtypes; hence, this PP-preferring subtype will not be discussed here. The y₆ receptor represents a non-functional pseudogene in humans and primates, is absent from the rat genome, and its pharmacological recognition profile remains controversial; hence, it will also not be discussed here.

Sequence comparisons show that receptors Y₁, Y₄ and y₆ are more closely related to each other than to the receptors Y₂ and Y₅. This is apparent not only from sequence identity but also from other features, such as cysteines believed to form disulfide bonds and the size of the third cytoplasmic loop, which is large in Y₅. The receptors Y₂ and Y₅ are equally distantly related to one another as to the Y₁/Y₄/y₆ group. In fact the Y₁/Y₄/y₆ group, the Y₂ and the Y₅ receptor are more distantly related to one another than any other G-protein-coupled receptors that bind the same endogenous ligand, despite the fact that Y₁, Y₂ and Y₅ each bind two distinct endogenous ligands, namely NPY and PYY. However, based on pharmacological recognition profiles, the receptors Y₁ and Y₅ are more similar to one another than to the receptor Y₄ (see below). Therefore, at present no formal division of NPY receptors into subfamilies is recommended.

- Y₁ receptors have been cloned from humans, rats, mice and from non-mammals such as *Xenopus laevis*. The genomic organisation of the Y₁ subtype gene has been determined in humans and mice, and the human gene has been located on chromosome 4q (31.3–32). Three splice variants in the 5' region of the human Y₁ receptor yield multiple promoters with tissue-specific expression patterns. Two splice variants of the murine Y₁ receptor have been described. While both variants bind NPY, the form with a shortened seventh transmembrane-spanning region and a lacking C-terminal tail does not appear to couple to signal transduction as efficiently as the full length form. Messenger RNA for the Y₁ receptor has been detected in a variety of human, rat and murine tissues including brain, heart, kidney and gastrointestinal tract.

- Y₂ receptors were originally cloned from human SMS-KAN cells. Later cloning studies in rats demonstrated that the cloned Y₂ receptor is also the molecular correlate of a previously proposed PYY-preferring receptor in the gastrointestinal tract. Messenger RNA for the Y₂ receptor has been detected in various parts of the CNS, whereas only low levels of Y₂ mRNA were found in human peripheral tissues.
- Y₅ receptors were cloned from humans and rats. Interestingly, the corresponding gene resides on human chromosome 4q in the same location as the human Y₁ receptor gene, but in opposite orientation. Messenger RNA for Y₅ receptors was detected by Northern blotting and in situ hybridisation in several rat brain areas, including those believed to be important for the regulation of food intake, as well as in testis.

All known NPY receptors belong to the large superfamily of **G-protein coupled receptors**. They appear to use similar signal-transduction pathways, and no clear and consistent alignment of a specific receptor type with a distinct transduction pathway has been identified. In almost every cell type studied (with the possible exception of some prejunctional receptors), NPY receptors act via pertussis toxin-sensitive **heterotrimeric GTP-binding proteins**, i.e. members of the G_i and G_o family. The typical signalling responses of NPY receptors are similar to those of other G_i/G_o-coupled receptors. Thus, inhibition of **adenylyl cyclase** is found in almost every tissue and cell type investigated and also with all cloned NPY receptor subtypes upon heterologous expression. Additional signalling responses that are restricted to certain cell types include inhibition of Ca²⁺ channels, e.g. in neurons, and activation and inhibition of K⁺ channels, e.g. in cardiomyocytes and vascular smooth muscle cells. Based on experiments with Ca²⁺ entry blockers, it has been postulated that NPY stimulates Ca²⁺ channels in the vasculature. In some cell types, members of the NPY family can mobilise Ca²⁺ from intracellular stores. While this appears to involve inositol phosphates in some cells, inositol phosphate-independent Ca²⁺ mobilisation has been postulated in other cell types. A sensitivity of certain responses to NPY to the cyclooxygenase inhibitor, indomethacin, indicates possible activation of a phospholipase A₂ by NPY receptors, but this has yet to be demonstrated definitively. Activation of a phospholipase D or of a tyrosine kinase, which can occur with some G_i/G_o-coupled receptors, has also not clearly been demonstrated. Thus, in general, Y receptors demonstrate a preferential coupling to pertussis toxin-sensitive G-proteins, i.e. the G_i and G_o family, which is followed by the responses typically under the control of these G-proteins.

Drugs

NPY receptors and NPY-induced responses were originally classified based on agonist orders of potency, but the advent of several subtype-selective antagonists has at least partly superseded the use of agonists for classification purposes. In some cases, however, particularly in vivo, agonists may still be required for receptor characterisation. This is based on the use of NPY, PYY, [Pro³⁴]-substituted analogues (which may or may not contain an additional [Leu³¹] substitution) and on C-terminal fragments of NPY and PYY (including the endogenous NPY_{3–36} and PYY_{3–36}).

Y₁ receptors are characterised by an agonist order of potency of NPY ≥ PYY ≥ [Pro³⁴]-substituted analogue >> C-terminal fragment > PP. C-Terminal fragments may act as partial agonists at Y₁ receptors and in some cell lines even as antagonists; whether such partial antagonism also occurs with intact tissues or in vivo remains to be determined. Y₁-selective antagonists include BIBO 3304 ((*R*)-*N*-[[4-(aminocarbonylaminoethyl)-phenyl]methyl]-*N*-(2-diphenylacetyl)-argininamide trifluoroacetate, *K*_i or *K*_B 0.2–1 nM), and BIBP 3226 ((*R*)-*N*-(2-diphenylacetyl)-*N*-[[4-(4-hydroxyphenyl)methyl]-arginine amide, *K*_i or *K*_B 1–10 nM), with BIBP 3435 ((*S*)-*N*-(2-diphenylacetyl)-*N*-[[4-(4-hydroxyphenyl)methyl]-argininamide) being a much less active stereoisomer, which can be used as an inactive control for the latter. Other Y₁ receptor antagonists include SR 120819A ((*R,R*)-1-(2-[2-(2-naphthylsulphamoyl-3-phenyl-propionamido)-3-[4-[*N*-(4-[dimethylaminomethyl]-*cis*-cyclohexylmethyl)amidinophenyl]propionyl]-pyrrolidine) or the polypeptide GR 231118 (also known as 1229U91 or GW1229, homodimeric Ile-Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-CONH₂), but the latter has also been reported to be an agonist at Y₄ receptors in some cases.

Y₂ receptors are characterised by an order of potency of NPY ≈ PYY ≥ C-terminal fragment >> [Pro³⁴]-substituted analogue > PP. BIIE 0246 ((*S*)-*N*-(1-[2-[4-[(*R,S*)-5,11-dihydro-6(6h)-oxodibenz[b,e]azepin-11-yl]-1-piperazinyl]-2-oxoethyl]cyclopentyl]acetyl)-*N*-[2-[1,2-di-hydro-3,5-(4H)-dioxo-1,2-diphenyl-3H-1,2,4-triazol-4-yl]ethyl]-argininamide) is a Y₂-selective antagonist with an affinity of 3 nM.

Y₅ receptors are characterised by an order of potency of NPY ≥ PYY ≈ [Pro³⁴]-substituted analogue ≈ NPY_{2–36} ≈ PYY_{3–36} >> NPY_{13–36}; rat PP had very low potency at the rat and human Y₅ receptor, while human and bovine PP had affinities similar to those of NPY and PYY. Y₅-selective antagonists include CGP 71683A (trans-naphthalene-1-sulfonic acid 4-[4-amino-quinazolin-2-ylamino]methyl]-cyclohexylmethylamide hydrochloride) with an affinity of 1 nM.

As additional tools, transgenic mice overexpressing NPY and knockout mice lacking NPY, Y₁ receptors or Y₅ receptors have been published.

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Neuropeptides

The neuropeptides are peptides acting as neurotransmitters. Some form families such as the tachykinin family with substance P, neurokinin A and neurokinin B, which consist of 11 or 12 amino acids and possess the common carboxy-terminal sequence Phe-X-Gly-Leu-Met-CONH₂. Substance P is a transmitter of primary afferent nociceptive neurones. The opioid peptide family is characterized by the C-terminal sequence Tyr-Gly-Gly-Phe-X. Its numerous members are transmitters in many brain neurones. Neuropeptide Y (NPY), with 36 amino acids, is a transmitter (with noradrenaline and ATP) of postganglionic sympathetic neurones.

- Neuropeptide Y
- Opioid Systems
- Synaptic Transmission

Neuropilins

Neuropilins are transmembrane proteins which bind to class 3 semaphorins as well as to vascular endothelial growth factor. The two mammalian neuropilins NRP-1 and NRP-2 were originally identified as important receptors for the neuronal guidance factors of the class 3 semaphorin family. Recently, increasing evidence has emerged implicating neuropilins in the regulation of angiogenesis as well as in tumour progression.

- Plexins

Neurosteroids

Neurosteroids are neuroactive steroids, which are synthesized in the brain. Neurosteroids can bind to and modulate the activity of γ -aminobutyric acid_A (GABA_A) receptors.

- GABAergic System
- Benzodiazepines

Neurotensin/Neuromedin N

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Definition

Neurotensin (NT) is a neuropeptide predominantly expressed in the brain and the gut. The peptide exerts its effects through two G-protein-coupled receptors, NTS1 and NTS2, and through a single transmembrane domain protein, NTS3 or sortilin, that belongs to a small family of sorting receptors. In the brain, NT is predominantly expressed in neurons within discrete brain areas and nuclei where it modulates the activity of neuronal systems involved in behavioral responses such as locomotor activity, rewarding, drug seeking, eating and pain sensation. In the gut, NT is primarily produced in endocrine N cells scattered throughout the jejuno-ileal mucosa and regulates a number of processes including gastrointestinal motility, pancreatic and biliary secretion and growth of normal gastrointestinal tissues.

Basic Characteristics

Biosynthesis, Release and Inactivation

Like all neuropeptides, NT is synthesized as part of a larger precursor that also contains ►neurodynin N (NN), a 6 amino acid ►neurotensin-like peptide (Table 1). Pro-NT/NN is processed in the regulated secretory pathway of neuroendocrine cells by prohormone convertases PC1, PC2 and PC5-A that belong to a larger family of proprotein convertases. Due to differential cleavage specificity and tissue distribution of the convertases, pro-NT/NN processing gives rise to approximately a 1:1 and a 5:1 ratio of NT over NN content in the brain and gut, respectively. The peptides are stored in secretory vesicles and released from neuroendocrine cells in a Ca²⁺-dependent manner. NT and NN actions are terminated by desensitization of the

receptors they activate and by degradation mechanisms that take place in the extracellular space. NT degradation is ensured by three Zn metallo-endopeptidases, namely endopeptidases 3.4.24.11, 3.4.24.15 and 3.4.24.16, whereas NN degradation is mainly effected by aminopeptidase M, a Zn metallo-exopeptidase.

NT Receptors

NTS1

Agonist stimulation of ►NTS1 receptor leads to ►phospholipase C activation through the ►heterotrimeric G-proteins Gq coupling in all systems examined. However, coupling to other G-proteins may occur in some systems. Thus, coupling to Gs leading to adenylyl cyclase activation in NTS1 receptor-overexpressing transfected cell lines and in the MIA PaCa-2 human pancreatic cancer cell line, and coupling to Gi/o leading to adenylyl cyclase inhibition in the N1E115 cell line or to ►phospholipase A2 (PLA2) activation in transfected CHO cells have been observed. NTS1 stimulation also activates more distal transduction pathways such as the mitogen-activated protein kinase (►MAPK) and serine/threonine protein kinase Akt pathways that are involved in the growth-promoting effects of NT in normal and ►cancer tissues. NTS1 signaling properties are recapitulated in Table 2. Agonist binding to NTS1 induces internalization of receptor-ligand complexes into NTS1-expressing cells via clathrin-coated pits. Upon acute agonist exposure, internalized NTS1 receptors are targeted to lysosomes for degradation whereas under prolonged agonist exposure, NTS1 is routed towards the perinuclear Trans-Golgi recycling compartment.

Sanofi-Aventis has developed a nonpeptide NTS1 antagonist, ►SR 48692 (Table 1), which binds with higher affinity to NTS1 than to ►NTS2 and has proven extremely useful for probing the functions associated with NTS1. These studies and recent investigations in knockout mice that do not express NTS1 suggest that most of the known effects of NT are attributable to NTS1 (Table 2). Another nonpeptide NTS1 antagonist, SR 142948A, with higher affinity for both NTS1 and NTS2 and less selectivity for NTS1 than SR 48692 has also been characterized.

NTS2

NTS2 corresponds to the NT receptor originally described as having lower affinity for NT than NTS1 and as being selectively recognized by ►levocabastine, a nonpeptide H1 histamine antagonist that has no affinity for NTS1. NTS2 shares about 60% homology with NTS1. It exhibits surprising species- and expression system-dependent pharmacological properties that are recapitulated in Table 2. Thus, in mNTS2-expressing *Xenopus* oocytes, NT, levocabastine and SR 48692 all behave as agonists. In CHO cells stably transfected with rNTS2, levocabastine and SR 48692 are agonists.

Neurotensin/Neuromedin N. Table 1 Structures and potential therapeutical uses of NT receptor ligands

Compound	Structure	Properties	Therapeutic potential
NT	pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH	Natural ligand of NT receptors	
NN	H-Lys-Ile-Pro-Tyr-Ile-Leu-OH	Natural ligand of NT receptors	
	[^{99m} Tc](NaHis)Ac-Lys-Ψ(CH ₂ NH)-Arg-Pro-Tyr-t-Leu-Leu-OH	Stable radiolabeled NT analog	Diagnostic and radiotherapeutic use in cancers
NT69L	NMe-Arg-Lys-Pro-L-neo-Trp-t-Leu-Leu-OH	Potent NT analog that crosses the blood–brain barrier	Antipsychotic; analgesic; anorectic
JMV 431	Boc-Arg-Arg-Pro-Tyr-Ψ(CH ₂ NH)Ile-Leu-OH	Selective ligand at NTS2 versus NTS1; inverse agonist at the constitutively active human NTS2	Analgesic
SR 48692		Nonpeptide antagonist at NTS1; agonist at NTS2	Inhibition of tumor growth; drug addiction treatment

NMe: *N*-methyl, *t*-Leu: *tert*-leucine, *L*-neo-Trp: (2*S*)-2-amino-3-(1*H*-4-indolyl)propanoic acid, Boc: *t*-butyl-oxycarbonyl, Ψ: pseudopeptide bond.

Neurotensin/Neuromedin N. Table 2 NTS1 and NTS2 signaling properties

Receptor subtype	Expression system	Transduction pathway	Agonist	Antagonist	Inverse agonist
NTS1					
Rat and human	All systems	Coupling to Gq, phoslolipase C activation,	NT	SR 48692	
		Increase in inositol phosphates and cytosolic [Ca ²⁺]	NN	SR 142948A	
		Activation of MAPK pathway			
	Transfected cell systems with high expression levels	Coupling to Gs, stimulation of cAMP production			
	Rat	Neuroblastoma N1E115 cell line	Coupling to Gi, inhibition of cAMP production		
	Stably transfected CHO cells	Coupling to Gi, production of ara-chidonic acid			
Human	Human pancreatic Mia PaCa-2 cell line	Coupling to Gs, stimulation of cAMP production			
NTS2					
Mouse	Xenopus oocyte	Activation of Ca ²⁺ -dependent Cl ⁻ conductance	NT		
			SR 48692		
			Levocabastine		
Rat	Stably transfected CHO cells	Increase in cytosolic [Ca ²⁺]	SR 48692		
			Levocabastine		
	Primary cultured cerebellar granule cells	Increase in cytosolic [Ca ²⁺]	SR 48692		
		Activation of MAPK pathway	NT		
			Levocabastine		
Human	Stably transfected CHO cells	Increase in inositol phosphates, cytosolic [Ca ²⁺], arachidonic acid; activation of MAPK pathway	SR 48692	NT	
			SR 142948A	Levocabastine	
	Transiently trans-fected COS cells	Constitutive activity	SR 48692	NT	Levocabastine
		Increase in inositol phosphates			JMV 431

In primary cultured rat cerebellar granule cells that endogenously express rNTS2, SR 48692 is agonist on the Ca response while NT and levocabastine are agonists on the MAPK pathway. Finally, in CHO cells stably transfected with hNTS2, both SR 48692 and SR 143948A are agonists whereas NT and levocabastine are antagonists. Interestingly, in another hNTS2-expressing system, i.e., transiently transfected COS cells, constitutive NTS2 activity is detected and SR 48692, levocabastine and NT behave as agonist, inverse agonist and neutral antagonist, respectively. NTS2 efficiently internalizes upon agonist exposure and recycles back to the membrane.

NTS3

►NTS3, also termed sortilin, is a 100 kDa protein that shares homology with the yeast vacuolar sorting protein Vps10p and other mammalian sorting receptors. This family of proteins is characterized by a single transmembrane domain, a cystein-rich domain, a signal peptide, a furin cleavage site and a short cytoplasmic tail. Sortilin/NTS3 has been cloned from the human and rat. This receptor might be involved in protein sorting between and from the cell surface to intracellular compartments. Binding of NT to NTS3 was reported to elicit proliferative responses in a variety of cancer cell lines and to alter agonist-induced cellular trafficking and signaling of NTS1 in HT29 human colon cancer cells. The strongest evidence that NTS3 participates in NT signaling comes from the demonstration that NTS3 mediates NT-induced migration of human microglial cells by a mechanism dependent on the stimulation of both MAPK and ►PI3-kinase pathways.

Neurotensin–Dopamine Interaction

Centrally administered NT exerts a number of effects similar to those of neuroleptic drugs. There is at present unquestionable neuroanatomical, electrophysiological, neurochemical and behavioral evidence that NT modulates dopamine (DA) transmission in the nigro-striatal and mesocorticolimbic pathways. Actually, NT behaves either as a ►psychostimulant or as a neuroleptic depending on what part of the mesocorticolimbic DA circuitry is targeted by the peptide. In vitro and in vivo application of NT to DA neurons of the ventral tegmental area (VTA) stimulates DA neuronal activity. In contrast, administration of NT in the nucleus accumbens, a projection area of the VTA, inhibits DA transmission. The stimulatory, psychostimulant-like effects are mediated through NTS1 receptors that are abundantly expressed by VTA DA neuronal cell bodies and dendrites. The inhibitory, neuroleptic-like effects are also mediated via NTS1 receptors located postsynaptically to DA nerve terminals in the VTA. In addition, both ►antipsychotic drugs and psychoactive drugs modify NT expression and transmission in

these circuits, suggesting that the peptide mediates the effects of both types of drugs. Recent studies with either NTS1 antagonist SR 48692 or with NT and NTS1 knockout mice further support the notion that NT participates, via NTS1, in the response to psychostimulants as well as to that of both typical and atypical neuroleptic drugs.

Nociception

Centrally administered NT in rodent elicits a potent, opioid-independent ►analgesic response. Recent studies show that the peptide exerts a biphasic modulation of ►nociception when injected in the rostral ventromedial medulla (RVM), low and high doses of peptide eliciting facilitatory and inhibitory effects, respectively. The recent demonstration that NT knockout mice display defects in stress-induced analgesia may suggest that the transition from pain facilitation to analgesia involves stress-induced increases in NT signaling in pain modulatory regions. Injection of SR 48692 in the RVM blocks the inhibitory but not the facilitatory effect of NT on nociception. This is in contrast with the report that SR 48692 failed to antagonize the analgesic response to NT in the rat and mouse following ip injection. These apparently discrepant data suggest that complex interactions occur between central NTergic systems and nociceptive circuits and that several NT receptor subtypes might be involved in mediating pain modulation by the peptide. Actually, recent evidence indicates that both NTS1 and NTS2 participate in the antinociceptive action of NT. Thus, NTS1 deletion in mice abrogates the analgesic effect of NT. On the other hand, partial reduction of central NTS2 expression in mice by means of icv administration of antisense oligonucleotides significantly decreases the antinociceptive effect of NT.

Feeding Behavior

A number of orexigenic and anorexigenic hypothalamic neuropeptides are known to coordinately regulate body weight through a complex, partially elucidated mechanism orchestrated in part by ►leptin, an adipose tissue-released hormone that acts centrally in the hypothalamus to control food intake and energy expenditure. The anorexigenic effect of centrally administered NT has been known for 20 years. Further support for a role of NT in food intake came from the observation that hypothalamic expression levels of NT are decreased in genetic models of obese rats and mice including the Zucker fa/fa rat and the ob/ob mouse that are deficient in leptin receptor and leptin, respectively. A number of recent reports point to NT as a possible mediator of leptin-induced reduction in food intake. Thus, leptin receptors are present on and activate NT-expressing neurons in the arcuate nucleus and the dorsomedial area of the rat

hypothalamus. Furthermore, central administration of leptin for 3 days increases NT gene expression whereas chronic intraperitoneal administration for 7 days decreases NT levels in the lateral hypothalamus. Leptin and NT reciprocally potentiate each other's inhibitory effect on food intake. Immunoneutralization of central NT with a neurotensin antiserum or administration of the NT antagonist SR 48692 completely block the inhibitory effect of leptin on food intake in food-deprived rats. Finally, in contrast to NT knockout mice that do not differ in their body weight from their wild type littermates, NTS1 knockout mice exhibit increased food consumption, body weight and white adipose tissue mass as compared to wild type littermates.

Drugs

In this part we shall examine the potential of NT receptors-targeting agents as therapeutic drugs.

Cancer

As mentioned above, NT exerts trophic effects on normal tissues. It is also well documented that the peptide stimulates the growth of cancer tissues. This is the case in colon, pancreas, lung, breast and prostate cancers. In general, NTS1 is ectopically expressed in these cancers and NT, through NTS1, activates mitogenic pathways that involve activation of MAP kinase and other nonreceptor tyrosine kinases. NT effects on cancer cell growth are generally inhibited by SR 48692. This compound could therefore have therapeutic value for the treatment of cancers.

NT agonists could also be useful for the treatment of cancer. We have seen that NTS1-agonist complexes are internalized in target cells. This has led to the development of suitably radiolabeled NTS1-targeting agonists with potential diagnostic and radiotherapeutic value. The primary concern in designing these compounds is to make them resistant to peptidases so as to maximize tumor/blood ratios. One such compound, which is in fact a technetium-radiolabeled NN analog, is shown in Table 1.

Schizophrenia and Drug Addiction

Clinical and postmortem data indicate that central NT systems are altered in schizophrenic patients. However, we have seen that NT could exert neuroleptic-like or psychostimulant effects depending on the site of administration in the rat. It has therefore been debated for many years whether a NTS1 agonist or antagonist could be used in the treatment of ►schizophrenia. Recent evidence points to NT agonists as offering promising leads for developing an active ►neuroleptic-like drugs. Thus, SR 48692 failed in clinical trials to reveal antipsychotic properties whereas NT agonist

mimetics that cross the blood–brain barrier, such as NT69L (Table 1), proved to exert neuroleptic-like effects in the rat following peripheral administration. On the other hand, there are reasons to believe that a NT antagonist might find useful application in treating drug addicts since ►amphetamine and cocaine ►cocaine- and amphetamine-regulated transcript sensitization are attenuated by SR 48692 and SR 142948A in the rat.

Pain Suppression

Data reviewed above support the involvement of NTS2 in mediating NT-induced analgesia. Interestingly, the analgesic potencies of icv injected metabolically stable NT analogs correlates positively with their affinities at NTS2 but not at NTS1. One of these analogs, JMV 431 (Table 1), is highly selective (100-fold higher affinity) for NTS2 versus NTS1 and exhibits high analgesic potency following icv administration. NT69L (Table 1) also induces analgesia following ip injection but its selectivity at NTS2 is not known. Further research to design NTS2-selective novel analogs that cross the blood–brain barrier may offer new avenues for the treatment of pain without the side effects associated with the use of opioids and devoid of NTS1-mediated effects.

Obesity

Evidence presented above strongly suggests that NT is a major link in the complex neuropeptide network that controls body weight and that the peptide effects on feeding are exerted through NTS1 receptor. NT agonists could therefore be useful in the treatment of obesity. In this context, it is of interest that NT69L (Table 1) was found to reduce food intake and body weight following ip administration in normal and genetically obese Zucker fa/fa rats.

- Phospholipases
- Transmembrane Signaling
- Heterotrimeric G-Proteins
- Nociception
- Analgesics
- Cancer
- Appetite Control

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Neurotransmitter Transporters

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Synonyms

Neurotransmitter transporters; Members of the family of solute carriers; Products of the (solute carrier) SLC1, SLC6, SLC17, SLC18, and SLC22 gene families

Definition

Neurotransmitter transporters create neurotransmitter gradients across membranes, which results in the uptake of the neurotransmitter. By working in reverse they can also release neurotransmitter into the extracellular space (efflux by nonexocytotic release).

Basic Characteristics

Common Features of Neurotransmitter Transport

Neurotransmitter transport is in principle coupled to charge transfer: a gradient of charged effective solutes (e.g., sodium ions) typically is the driving force. As building this gradient consumes energy, neurotransmitter transporters are classified as secondary-active carriers. Alternatively, transporters may mediate the electroneutral exchange of two substrates (antiport).

Substrate specificity is determined by high affinity for the cognate neurotransmitter substrate. However, low affinity uptake does also have a part in the clearance of transmitters from the interstitial space (e.g., in uptake mediated by the extraneuronal monoamine transporter, EMT) and in the intestinal absorption of glycine and glutamate. It is obvious that there is an evolutionary relation of neurotransmitter transporters and amino acid and cation transporters in epithelia.

Neurotransmitter transporters fulfill three fundamental functions.

1. Transport supplies nerve cells with neurotransmitter, which is used to replenish secretory vesicles, thus uptake occurs on the cell surface and on the secretory vesicle.

2. Neurotransmitter transporters determine the neurotransmitter concentration in the interstitium. High-affinity transporters can efficiently remove neurotransmitter from the extracellular space because cellular uptake is typically coupled to the translocation of sodium ions.
3. Neurotransmitter transporters terminate the time interval of synaptic neurotransmission; localization of transporters in the vicinity of exocytotic sites is crucial for their clearance of neurotransmitter molecules following their exocytotic release into the synaptic cleft.

While these functions can be carried out by a single transporter isoform (e.g., the serotonin transporter, SERT) they may be split into separate processes carried out by distinct transporter subtypes, or in the case of acetylcholine, by a degrading enzyme. Termination of cholinergic neurotransmission is due to **▶acetylcholinesterase** which hydrolyses the ester bond to release choline and acetic acid. Reuptake of choline into the nerve cell is afforded by a high affinity transporter (CHT of the SLC5 gene family).

Loss or gain of transport function causes disorders: low levels of monoamine neurotransmitters are associated with depression, uptake inhibition can result in the spill-over of glutamate to neighboring synapses and **▶excitotoxicity**. Glutamate can even be released (by efflux) in large amounts if ion gradients can no longer be maintained due to impaired energy metabolism (as in the case of brain ischemia). In keeping with the potentially noxious effects of glutamate, animal experiments have suggested that enhanced removal of glutamate increases resistance to neurodegeneration in **▶amyotrophic lateral sclerosis**.

Structure

The molecular structure of the glutamate SLC1 (**Table 1**) and the monoamine SLC6 transporters (**Table 2** and **3**) differ remarkably. The glutamate transporters are assembled of three integral membrane proteins to form a bowl embedded in the membrane; each protomer has a specialized hairpin peptide domain to bind and carry the substrate (glutamate and ions). The monoamine transporter protomer is anchored in the membrane with 12 transmembrane spans (relative to eight in the glutamate transporter) but the structure does not disclose how the substrate is translocated. Monoamine transporters also assemble as homo-oligomers; while their quaternary structure likely has evolved to ensure surface targeting of the transporters, the oligomeric state also may be a prerequisite for amphetamine induced efflux.

Turnover of Neurotransmitter at the Synapse

Neurotransmitter turnover differs for the individual neurotransmitters. Glutamate and glycine require

Neurotransmitter Transporters. Table 1 SLC1 family transporters for L-glutamate (and D/L-aspartate)

Transporter (gene)	Distribution	Km ($\mu\text{mol/l}$)	Major Function
EAAC1/EAAT3 (SLC1A1)	Neurons in many brain regions	25–50	Limits glutamatergic signaling
	Kidney epithelium		Resorption of dicarboxylic amino acids
GLT1/EAAT2 (SLC1A2)	Astrocytes in cerebral cortex, hippocampus	5–100	Clearance of interstitial glutamate
GLAST/EAAT1 (SLC1A3)	Glial cells in cerebellum, retina, inner ear	5–70	

Neurotransmitter Transporters. Table 2 SLC6 family transporters for GABA (γ -amino butyric acid) and glycine

Transporter (gene)	Distribution	Km ($\mu\text{mol/l}$)	Major function	Selective inhibitor
GABA transporter GAT1 (SLC6A1)	GABAergic neurons in CNS	≤ 10	Clearance of interstitial neurotransmitter, reuptake into neurons	Tiagabine, an antiepileptic drug
Glycine transporter GlyT1 (SLC6A9)	CNS-glia	~ 20	Clearance of interstitial neurotransmitter	Sarcosine
GlyT2 (SLC6A5)	CNS-neurons		Reuptake into neurons	None

Neurotransmitter Transporters. Table 3 SLC6 family transporters for monoamines

Transporter (gene)	Substrate	Km ($\mu\text{mol/l}$)	Distribution	Function
NET (SLC6A2)	Noradrenaline, dopamine	~ 1	CNS noradrenergic neurons (emanate from locus coeruleus and lateral tegmental area), sympathetic nervous system	Clearance of interstitial neuro-transmitter, reuptake into neurons
DAT (SLC6A3)	Dopamine	~ 5	CNS dopaminergic neurons (emanate from substantia nigra, other midbrain nuclei, hypothalamus)	
SERT, 5HTT (SLC6A4)	5HT = serotonin	~ 1	CNS serotonergic neurons (emanate from raphe nuclei) platelets, smooth muscle, intestine	

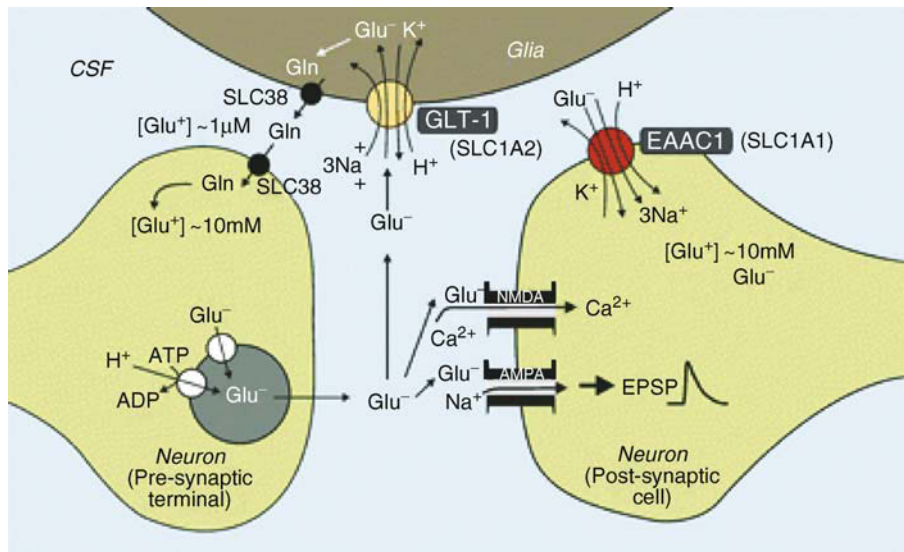
nonneuronal cells equipped with distinct transporters for control of their interstitial concentration, whereas dopamine, noradrenaline, and serotonin do not.

Figure 1 depicts a glutamatergic synapse. At a presynaptic terminal l-glutamate is stored in secretory vesicles equipped with vesicular glutamate transporters (VGluT1/2). Upon release glutamate activates ionotropic receptors (▶AMPA receptors and ▶NMDA receptors). The figure shows the glial glutamate transporter GLT1 and the neuronal glutamate transporter EAAC1 present on the postsynaptic neuron. Because there is no glutamate transporter on the presynaptic cell its supply with glutamate therefore must be indirect. Much of the glutamate accumulated in glial cells (GLT1 or GLAST) is metabolized to glutamine. Transport of glutamine that has no effect on neurotransmitter receptors occurs through a distinct carrier system (SLC38). Synthesis of glutamate in the nerve cell is then catalyzed by glutaminase, an

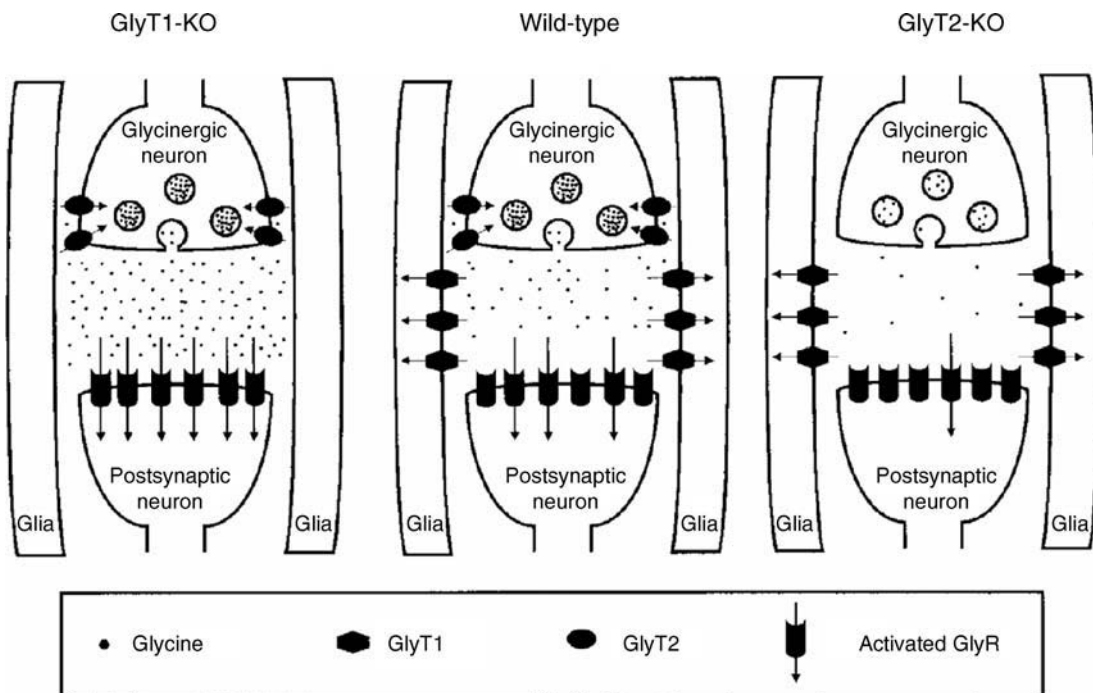
enzyme that also occurs in the kidney where it catalyzes the reverse reaction to deliver glutamate and ammonia into the urine.

In the brain glutamate is present at high levels and is mostly intracellular. The level is about thousand-fold higher than that of the monoamine neurotransmitters. It is reasonable to assume that the glial cells, which outnumber the nerve cells, have evolved to detoxify the brain's glutamate load and control excitotoxicity; because they do not store but metabolize glutamate, glial cells expressing glutamate transporters contain less glutamate than glutamatergic neurons.

Figure 2 shows that handling of glycine follows a similar principle. Glycine is a major inhibitory neurotransmitter but in addition it is essential for glutamatergic neurotransmission by serving as co-agonist at the NMDA-receptor. Removal of glycine from the synaptic space is due to uptake by GlyT1 on neuroglia (astrocytes), which is



Neurotransmitter Transporters. Figure 1 Glutamate transporters at a glutamatergic synapse. Upon exocytotic release of glutamate neurotransmission occurs by binding to and activation of ionotropic receptors. AMPA-receptors mediate fast excitatory postsynaptic potentials (EPSP), NMDA-receptors mediate slower calcium influx. Transmission is terminated by desensitization of the receptors and by glutamate diffusion out of the synaptic cleft; glutamate spillover is limited by uptake into the postsynaptic neuron (EAAC1) and into astrocytes (GLT1). (From; Kanai Y, Hediger MA. *Pflügers Arch* (2004) 447:469–479)



Neurotransmitter Transporters. Figure 2 Differential role of the glycine transporter isoforms, according to experiments where GlyT1 and GlyT2 were inactivated. The neuronal and glial location of GlyT1 and GlyT2 are indicated. In the absence of GlyT1 (GlyT1-KO) glycinergic neurotransmission is potentiated. With GlyT2 being inactivated (GlyT2-KO) neurotransmission decreases. (From Gomez J, Arnsen W, Betz H, Eulenburg V: (2006) *Neurotransmitter Transporters, Handbook of Experimental Pharmacology* (175):457–483. Lessons from the knocked-out glycine transporters.)

responsible for limiting the intensity and duration of glycinergic neurotransmission while GlyT2 replenishes the presynaptic nerve cell (see also Table 2). GlyT2 therefore is a marker of glycinergic nerve terminals which are abundant in the spinal cord, brainstem and the cerebellum. Inactivation of GlyT2 results in muscular spasticity and is lethal; tetanus toxin which specifically blocks neurotransmitter exocytosis from glycinergic interneurons in the spinal cord has a similar effect. The distribution of GlyT1 mostly overlaps with that of GlyT2 but GlyT1 is also present in higher brain regions.

The transporters for 5HT, noradrenaline and dopamine, biogenic monoamines, are genetically related, exist as single isoforms and are expressed on the surface of nerve cells, which use monoamines as (or convert them into) their cognate neurotransmitter. The single-isoform monoamine transporters fulfil all three fundamental functions (reuptake, limiting synaptic transmission, and control of the extracellular neurotransmitter concentration). Inactivation of DAT, NET, or SERT results in an increased extracellular lifetime and level of monoamine neurotransmitter, but decreased intracellular storage and evoked release (Fig. 3).

DAT is predominantly expressed by dopaminergic brain neurons, NET by noradrenergic neurons in the central and peripheral nervous system, and SERT is restricted to the axons of serotonergic neurons, which originate in the ▶**raphe nuclei** and innervate numerous higher brain regions; therefore SERT is widely distributed in the brain. Outside the brain, 5HT transport can be measured on non-neuronal cells (e.g. platelets, lymphoblastoid cells and smooth muscle cells); most of the 5HT appearing in the circulation is taken up by platelets.

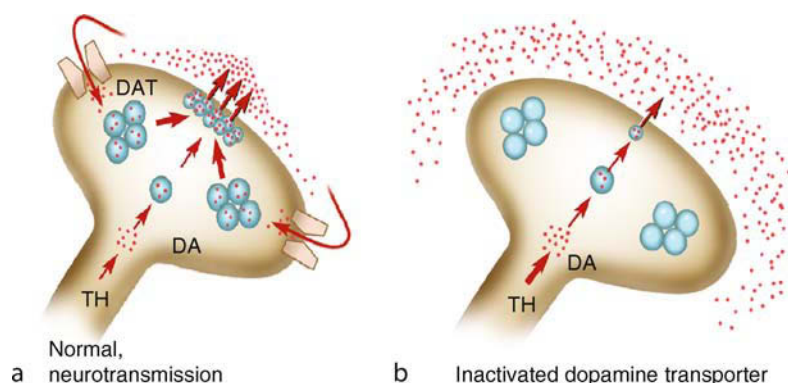
In the nervous system, DAT, NET, and SERT are distributed along axons, soma and dendrites. On the subcellular level they are localized at the

peri-synaptic plasma membrane – as opposed to a synaptic localization – indicating that the neurotransmitter diffuses out of the synaptic cleft to be transported back into the nerve cell (Fig. 3); alternatively, the monoamine transmitter may travel longer distances to be taken up far from the release site, possibly by a different nerve cell; the associated form of long-range neurotransmission is called volume transmission.

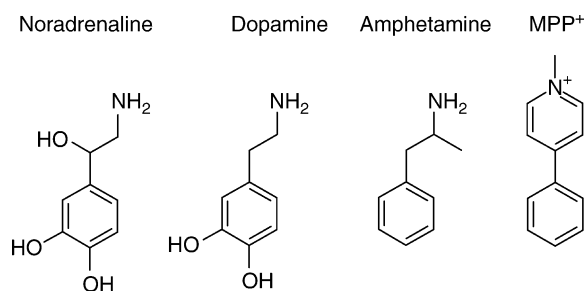
Ion Coupling Requirements

Most of the neurotransmitter transporters transform gradients of charged solutes (ions) into energy needed to pump neurotransmitters across the membrane. For instance, the glutamate carrier moves three Na^+ ions and one H^+ ion to the inside and 1 K^+ ion to the outside, whereas monoamine transporters carry along one to two Na^+ and one Cl^- ion. By contrast, vesicular transporters accumulate monoamine substrate at the expense of proton antiport (as the interior of the secretory vesicles is acidic); vesicular glutamate uptake additionally requires an electrical gradient. Extraneuronal monoamine transport is different as it is independent from the sodium gradient but only needs a positively charged substrate molecule.

Neurotransmitter transport can be electrogenic if it results in the net translocation of electrical charge (e.g. if more cations than anions are transferred into the cell interior). Moreover, some transporters may directionally conduct ions in a manner akin to ligand-gated ion channels; this ion flux is not coupled to substrate transport and requires a separate permeation pathway associated with the transporter molecule. In the case of the monoamine transporters (DAT, NET, SERT) the sodium current triggered by amphetamine, a monoamine and psychostimulant (see Fig. 4) is considered responsible for a high internal sodium concentration



Neurotransmitter Transporters. Figure 3 Dopamine turnover at a presynaptic nerve terminal. (a) Dopamine is produced by tyrosine hydroxylase (TH). When secretory vesicles are filled, they join the releasable pool of vesicles at the presynaptic membrane. Upon exocytosis, the diffusion of released dopamine is limited by reuptake via DAT. (b) If DAT is inactive, dopamine spreads in the cerebrospinal fluid but cannot accumulate in secretory vesicles. This results in a compensatory increase of dopamine hydroxylase activity and a higher extracellular dopamine level: mice with inactive DAT are hyperactive.



Neurotransmitter Transporters. Figure 4 The structures of DAT/NET substrates share an amino group linked to a phenyl ring by a chain of carbon atoms. MPP⁺ the metabolite of the neurotoxin MPTP (Methyl -phenyl-tetrahydropyridine) is also a substrate for the extraneuronal monoamine transporter.

that allows for neurotransmitter efflux. Amphetamine, in addition, sets free the vesicle content into the cytosol thus providing substrate for efflux; amphetamine molecules are transported by vesicular transporters and because they are weak bases dissipate the pH gradient, which is necessary to maintain high intravesicular substrate concentrations. Hence their effect is diminished by reserpine, an inhibitor of the vesicular monoamine transporter. The use of reserpine depletes the central nervous system of monoamine neurotransmitter, which causes akinesia and depression and in addition lowers sympathetic outflow leading to reduced blood pressure.

Regulation of Transporter Surface Expression

The localization of transporter molecules on the cell surface is dynamic rather than constitutive, such that transport capacity may be adapted to neuronal activity. Obviously, the mechanisms regulating uptake are of principal importance in pharmacology just as pharmacological transport inhibitors can regulate the density of transporters.

There are examples that cells have an intracellular reserve of functional transporter moieties: EAAC1, GAT1, CHT are predominantly – while NET only in some nerve cells – localized in the cell interior where transporters are stored membrane-bound in vesicles. Regulation occurs by transporter redistribution. Increased surface density leads to increased transport capacity while transporter internalization suppresses uptake.

The most prominent way a cell regulates transporter redistribution is via phosphorylation events although it is not clear if phosphorylation of the transporter protein itself is critically involved. High-affinity monoamine transport as well as transport by GAT1 and GlyT2 (SLC6A family members) is diminished upon activation of protein kinase C. PKC elicits internalization of transporter molecules and this is also true for the glutamate transporter (GLT1). By contrast, the

neuronal glutamate transporter EAAC1 increases upon PKC activation due to transporter redistribution from the cell interior to the surface. Since the plasma membrane residency of an EAAC1 transporter molecule is short (in the range of minutes) fast recycling of transporters must occur in a constitutive fashion; the action of PKC appears to slow internalization.

The **PKC family of protein kinases** comprises an important family of cellular signal transducing molecules; its members may be stimulated by a rise in free intracellular calcium and by esters of fatty acids (such as diacylglycerol), which are products of **phospholipase C**. Thus, PKC is stimulated indirectly by many receptors (G-protein coupled receptors and receptor tyrosine kinases) or directly by signals that cause calcium influx. In addition, transportable substrates of DAT (dopamine, amphetamines) can also trigger internalization by a mechanism that relies on PKC activation. This is not a general phenomenon among SLC6 family members but may represent a specific neuroprotective mechanism that limits the intracellular accumulation of toxic dopamine metabolites.

A second mechanism that impinges on the localization of transporters is through the association with proteins, the most prominent example being syntaxin. Syntaxin is a t-SNARE protein necessary for the fusion of vesicles with the plasma membrane (see the chapter on exocytosis). On the cell surface syntaxin consistently stabilizes the localization of GABA, noradrenaline, glycine, and 5HT transporters; the PKC α isoform can sever the interaction with syntaxin suggesting a general mechanism for transporter internalization.

Drugs

Glutamate Transporters

Since glutamate is quintessential in most if not all brain functions drugs inhibiting glutamate transport would likely cause detrimental effects. Conversely, increasing glutamate transporter density can have neuroprotective effects in degenerative disorders such as amyotrophic lateral sclerosis (ALS) provided that the disorder is not caused by impaired energy metabolism; the latter might result in transport reversal and glutamate efflux. Increased expression of GLT1 was found to be induced by β -lactam antibiotics and a neuroprotective effect of these drugs has been demonstrated in models of glutamate neurotoxicity.

Monoamine Transporters

The original monoamine hypothesis of depression states that “depressions are associated with a deficiency of catecholamines, particularly norepinephrine, at functionally important adrenergic receptor sites in the brain. Elation conversely may be associated with an excess of such amines.” The hypothesis was articulated in 1966 only after the mechanism of action of the tricyclic antidepressant desipramine and of the psychostimulants

cocaine and amphetamine had been clarified. In principle, the SERT, NET, and DAT are targets for drugs with antidepressant effects; dopamine uptake inhibition, however, has invariably been associated with addiction and none of the available antidepressants is a potent inhibitor of DAT (see Table 4).

Cocaine and desipramine inhibit the reuptake of monoamine neurotransmitters whereas amphetamine, which is a phenylalkylamine – similar in structure to the catecholamines, see Fig. 4 – competes for uptake and more importantly, evokes efflux of the monoamine neurotransmitters. All of them exert antidepressant effects. Cocaine and amphetamine are addictive whereas tricyclic antidepressants and their modern successors are not. The corollary of the addictive properties is interference with DAT activity. Blockade of DAT by cocaine or efflux elicited by amphetamine produces a psychostimulant effect; despite the different mechanisms even the experienced individual can hardly discern their actions. Because of the risk associated with inhibiting DAT mediated dopamine clearance the antidepressant effects of psychostimulants has not been exploited.

There is no definitive answer to whether inhibition of noradrenaline or serotonin is the most important in conferring antidepressant efficacy. The fact that all drugs require a period of several weeks before they become fully effective suggest that they modify gene expression in the brain and that the resulting altered biochemical state takes a long time to become stabilized. Although the selective serotonin reuptake inhibitors (SSRI) are no more efficacious than the first-generation tricyclic antidepressants, and do not act any faster, they are considerably safer in overdose. The toxicity of tricyclic antidepressants is due to their binding to cellular structures other than NET/SERT (such as ion channels and receptors).

Treatment of ▶attention deficit hyperactivity disorder (ADHD) in children with psychostimulants

(amphetamine, methylphenidate) has often been labeled paradoxical, since these drugs are known to increase motor activity in healthy animals. Yet, these drugs effectively decrease motor excess in children with ADHD, improve symptoms and do so by increasing extracellular dopamine. The molecular targets are the dopamine carriers DAT and NET; abuse liability can be reduced with oral slow-release formulations of the psychostimulants or with the use of NET inhibitors (atomoxetine).

Undesired Effects of Drugs that Interfere with Transporter Function

The party drug MDMA (3,4-methylene-dioxymethamphetamine) as well as amphetamine causes efflux of all monoamine neurotransmitters. The effects of MDMA are described as psychostimulant and hallucinogenic and are judged differently from those of amphetamine. This difference is due to the stronger inhibition of SERT by MDMA as compared with amphetamine, which is a more potent dopamine releaser and more addictive than MDMA.

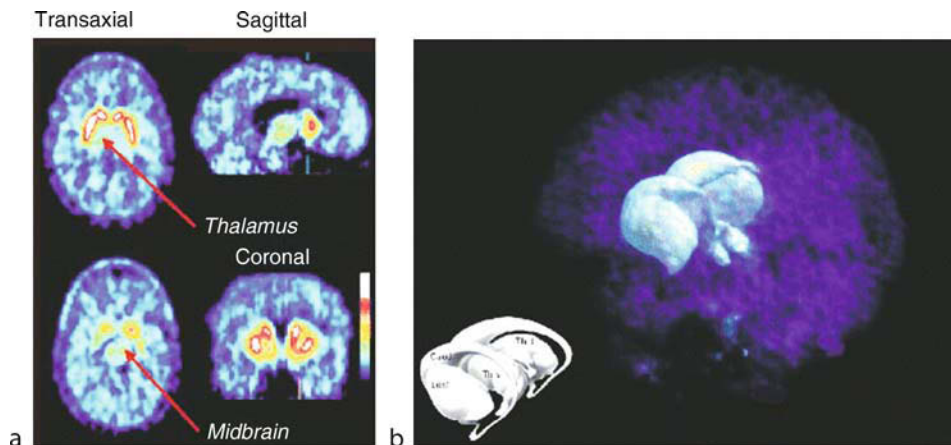
MDMA overdose as well as the concomitant consumption of selective serotonin reuptake inhibitors (SSRI) with other drugs that exert serotonergic effects (such as inhibitors of monoamine oxidase) can rapidly lead to the serotonin syndrome. Its symptoms, which are reversible upon cessation, of the drug include confusion, muscle rigidity in the lower limbs, and hyperthermia suggesting an acute reaction to serotonin overflow in the CNS. Blocking the function of SERT outside the brain causes side effects (e.g., nausea), which may be due to elevated 5HT; however, impairment of transporter function is not equivalent to direct activation of 5HT receptors in causing adverse effects such as fibrosis and pulmonary hypertension.

Due to its expression in the sympathetic nervous system NET activity is important in limiting the effects of

Neurotransmitter Transporters. Table 4 Clinically useful inhibitors of monoamine neurotransmitter transporters

Transporter	Moderately selective inhibitors in clinical use	Highly selective inhibitors (≥50-fold)	Nonselective inhibitors
NET	<i>Desipramine</i>	<i>Maprotiline, mianserin</i>	<i>Amoxapin, doxepine, nortriptyline, duloxetine</i>
	Reboxetine		
	Atomoxetine		
SERT	<i>Amitriptyline</i>	<i>Clomipramine</i>	
	<i>Imipramine</i>	Citalopram	
		Fluoxetine	
		Paroxetine	
		Venlafaxin	
DAT	Bupropion	–	–

Older tricyclic antidepressants are set in italics. The specificity of action of *tricyclic antidepressants* (in particular of *amitriptyline, imipramine, doxepine, nortriptyline, maprotiline*) is limited because at therapeutic levels these drugs also block receptors (H₁-histamine, α₁-adrenergic, muscarinic).



Neurotransmitter Transporters. Figure 5 PET images showing the brain distribution of DAT in an adult subject. Transaxial projections are at the level of the basal ganglia and at the upper midbrain. (b) Volume-rendered PET images. Clearly visible is the binding to the caudate (*Caud*) and putamen (*Lent*) structure. The midbrain structure (arrow) is the structure behind and below caudate-putamen. Insert: schematic drawing of striatum and thalamus (*Th s* and *Th d*, left and right side, respectively). (From: Jucaite A et al., *Eur J Nucl Med Mol Imaging* (2006); 33(6):657–668)

noradrenaline in the circulation. Thus amphetamines and cocaine can lead to vasoconstriction, high blood pressure, tachycardia, and myocardial ischemia resulting in chest pain. Conversely, NET malfunction due to a genetic effect is a cause of orthostatic intolerance without hypotension. If the metabolic decay of noradrenaline is blocked the susceptibility to transport reversal increases. Under this condition the natural food ingredient tyramine (present in wine and cheese) exerts a peripheral amphetamine-like action and may lead to a hypertensive crisis (“cheese syndrome”).

Age-related neurodegeneration primarily affects dopaminergic neurons projecting from the substantia nigra to the corpus striatum. DAT activity accounts for the vulnerability of dopaminergic neurons, as dopamine metabolites in the nerve cell create oxidative stress, which reduces viability. (i) DAT is necessary for high concentrations of intracellular dopamine; (ii) MPP⁺ the active neurotoxic principle of MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridin) is an excellent substrate of DAT (see Fig. 3). MPTP ingestion causes a Parkinson’s disease-related movement disorder of rapid onset. (iii) DAT is also a prerequisite for the brain damage caused by methamphetamine.

Although the corpus striatum is the area with the highest density of DAT in the brain, other brain regions can also succumb to the dopamine-related neurodegeneration (cognitive defects but not movement disorders are consequences of chronic methamphetamine abuse). In order to assess the severity of Parkinson’s disease cocaine analogues labeled with short-lived radioactive isotopes may be employed to quantify the density of dopaminergic nerve cells in vivo using positron emission tomography (PET) scanners. A decrease in imaging of the caudate nucleus/putamen (corpus

striatum) correlates in particular with bradykinesia and rigidity symptoms (an image from a healthy brain is shown in Fig. 5). From Fig. 5 it also becomes clear that the sensitivity of a PET scan is too limited to examine regions expressing DAT at low density.

Older tricyclic antidepressants are set in italics. The specificity of action of *tricyclic antidepressants* (in particular of *amitriptyline*, *imipramine*, *doxepine*, *nortriptyline*, *duloxetine*, *maprotiline*) is limited because at therapeutic levels these drugs also block receptors (H₁-histamine, α_1 -adrenergic, muscarinic).

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Neurotransmitters

Neurotransmitters are molecules that convey a signal from one nerve cell to the other. Neurotransmitters can be biogenic amines (e.g. norepinephrine, serotonin),

amino acids (e.g. γ -aminobutyric acid, glutamate) or neuropeptides (e.g. corticotropin releasing hormone, substance P).

► Synaptic Transmission

Neurotrophic Factors

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Synonyms

Neurotrophins

Definition

Neurotrophic factors are operationally defined as molecules that support neuron survival during development and subsequent to lesions. During development a significant portion of neurons undergoes apoptosis, the extent of which is widely believed to be controlled by limiting amounts of trophic factors provided by target tissues. Neurotrophic factors considered here include members of the nerve growth factor (NGF) family, the glial cell line-derived neurotrophic factor (GDNF) family, the ciliary neurotrophic factor (CNTF) family, as well as some members of the fibroblast growth factor (FGF) and transforming growth factor- β (TGF- β) families.

Mechanism of Action

NGF Family (Neurotrophins)

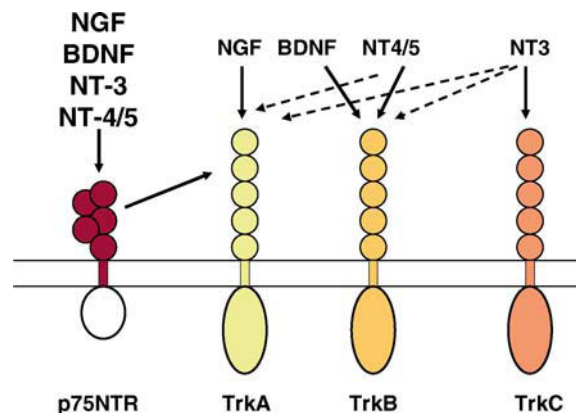
Neurotrophins (NGF; brain-derived neurotrophic factor, BDNF; neurotrophin-3, NT-3; NT-4; NT-6) are important regulators of neural survival, development, function, and plasticity of the vertebrate nervous system [1]. Neurotrophins generally function as noncovalently associated homodimers. They activate two different classes of receptors, through which signaling pathways can be activated, including those mediated by Ras and members of the *cdc42/rac/rho* G protein families, MAP kinase, PI-3 kinase, and Jun kinase cascades.

NGF binds to the transmembrane receptor tyrosine kinase (trk, or p140^{trk}), now referred to as TrkA. BDNF binds to TrkB, whereas NT-3 can bind to all three Trk (A,B,C) receptors, with a preference to TrkC, and NT-4/5 can bind both TrkA and TrkB. Furthermore, all neurotrophins also bind with equal affinity to a 75 kD transmembrane glycoprotein, p75^{NTR} (also referred to

as low affinity receptor). Pro-NGF can activate p75^{NTR} and induces cell death in the presence of the coreceptor sortilin (Fig. 1).

Neurotrophin binding leads to autophosphorylation of the cytoplasmic tyrosine kinase domain of trk, containing 10 conserved tyrosine residues. Three of them (Y670, Y674, Y675) are present in the autophosphorylatory loop that controls kinase activity, whereas the others create docking sites for adaptor proteins containing phosphotyrosine-binding (PTB) or src-homology-2 (SH-2) motifs. These adaptor proteins couple Trk receptors to intracellular signaling cascades, which include the Ras/ERK protein kinase pathway, the phosphatidylinositol-3-kinase (PI-3-kinase)/Akt kinase pathway, and phospholipase C (PLC)- γ 1. Neurotrophins induce rapid ruffling and cytoskeletal rearrangements involving small G proteins of the Cdc-42/Rac/Rho family, which regulate the polymerization and turnover of F-actin. The ability of Trk receptors to activate specific signaling pathways is regulated by membrane trafficking. Transmission of the signals from the nerve terminal to neuronal cell bodies requires retrograde transport of, e.g., NGF together with the activated Trk receptors in endocytotic vesicles, whereby membrane sorting is thought to determine which pathways are activated by Trk receptors. Tyrosine kinase-mediated signaling by endogenous Trk receptors has been shown to promote survival and differentiation of all respective neuron populations.

Several signaling pathways are also activated via the p75^{NTR} receptor. Intracellularly, p75^{NTR} interacts with proteins including TRAF6, RhoA, NRAGE, and NRIF, and regulates gene expression, cell cycle, apoptosis, mitogenic responses and growth cone motility. An important pathway promoting cell survival of many cell populations involves activation of NF κ B. All neurotrophins have been shown to promote association of p75^{NTR} with the adaptor protein TRAF-6, whereas only NGF seems to be able to induce nuclear translocation



Neurotrophic Factors. Figure 1 Neurotrophins.

of NF κ B. Binding of neurotrophins to p75^{NTR} has also been shown to activate the Jun kinase pathway.

GDNF Family

GDNF is described as a survival promoting and neuroprotective activity for mesencephalic dopaminergic neurons in vitro and in vivo, as well as for spinal motoneurons [2]. Gene targeting has revealed that GDNF signaling is also required for the development of the enteric nervous system and kidney morphogenesis. GDNF utilizes a receptor system comprised of a signaling component encoded by the c-ret protooncogene and a glycosylphosphatidylinositol (GPI)-anchored coreceptor, GDNF family receptor α 1 (GFR α 1), which is required for ligand binding. The GDNF family comprises four members, all of which utilize Ret as signaling receptor with the aid of different members of the GPI-linked coreceptor: neurturin-GFR α 2, artemin - GFR α 3, and persephin - GFR α 4, although promiscuity between the different receptors is also possible.

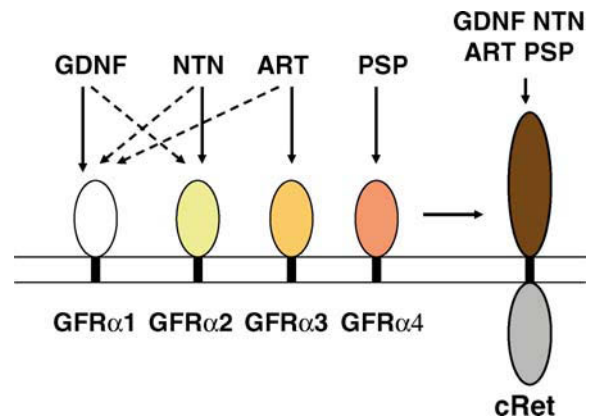
The current model of GDNF signaling proposes a rather stringent division in the functions of Ret and the GFR α receptor, whereby Ret is regarded as the signaling receptor and GFR α as the ligand-binding receptor. Signaling is initiated upon formation of the heterodimeric RET/GFR α receptor complex. In this context, GDNF-induced signaling leads to autophosphorylation of the intracellular tyrosine kinase of RET, the involvement of Grb2 adaptor and Shc docking proteins resulting in activation of the Ras/ERK, PI-3 kinase/Akt pathways as well as PLC- γ .

Recent studies have shown that GFR α receptors are localized in lipid rafts of the plasma membrane. The binding of GDNF to GFR α 1 also recruits Ret to the lipid rafts and triggers an association with src, which is required for effective downstream signaling. In absence of RET neural cell adhesion molecule NCAM can serve as a signaling receptor for GDNF in combination with GFR α 1, resulting in activation of Fyn and FAK protein tyrosine kinases. Furthermore, GDNF, ARTN and NRTN but not PSP have been shown to bind to heparin sulfate proteoglycan syndecan 3 serving as a coreceptor by presenting the ligand to the GFL/RET complex (Fig. 2).

CNTF Family

CNTF is expressed in glial cells within the central and peripheral nervous system. CNTF lacks a signal sequence and is not secreted by the classical secretory pathway, but is thought to convey its cytoprotective effects after release from adult glial cells by some mechanism induced by injury [3,5].

CNTF supports survival and differentiation of selected neuron populations including sensory, sympathetic, and motoneurons. Also, nonneuronal cells, such as



Neurotrophic Factors. Figure 2 GDNF family.

oligodendrocytes, microglial cells, liver cells, and skeletal muscle cells, respond to exogenous CNTF. Mice lacking CNTF develop normally and only in adulthood do they exhibit a mild loss in motoneurons, suggesting that CNTF acts on the maintenance of these cells [5].

The CNTF receptor complex is most closely related to, and shares subunits with, the receptor complex for interleukin-6 (IL-6) and leukemia inhibitory factor (LIF). The specificity conferring α subunit of the CNTF receptor complex (CNTFR α) is a GPI-anchored membrane protein lacking a conventional transmembrane domain. Mice lacking CNTFR α die perinatally, suggesting a developmentally important CNTF-like ligand. CNTF binding to CNTFR α results in the formation of a heteromeric tripartite receptor complex upon recruitment of gp130 and LIFR β . IL-6 requires IL6R α and a homodimer of gp130 for activity, and LIF requires gp130 and LIFR β . Signal transduction is mediated via tyrosine phosphorylation through constitutively associated Janus kinases (JAK) and signal transducers and activators of transcription (STAT). Activated STATs dimerize, translocate to the nucleus to bind specific DNA sequences and resulting in enhanced transcription of responsive genes.

FGF Family

FGFs have been demonstrated to influence the growth and function of cells of the vascular, muscular, epithelial and nervous systems. They are now thought to be involved in processes ranging from morphogenesis, tissue maintenance, and repair to oncogenesis. To date, 20 distinct FGFs have been discovered, numbered consecutively from 1 to 20. Particularly FGF2, FGF5, FGF9, and FGF20 have been discussed in the context of regulating neuron survival of, e.g., motoneurons, mesencephalic dopaminergic neurons, cholinergic neurons. FGFs are small polypeptide growth factors, many of which contain signal peptides for secretion. FGFs have a strong affinity for heparin and heparan-like glycosaminoglycans (HLGAG) of the extracellular

matrix. There are four FGF receptors, FGFR-1 to FGFR-4, known, containing two intracellular tyrosine kinase domains. FGFR diversity is additionally increased by alternative splicing of the individual FGFR genes. FGF signaling is propagated via PLC γ , src, Crk-mediated, and SNT-1/FRS2 signaling pathways.

TGF- β Family

TGF- β s are a growing superfamily of cytokines with widespread distribution and diverse biological functions [4]. TGF- β has been described to control neuronal performances including the regulation of proliferation of neuronal precursors, induction/specification of distinct neuronal phenotypes, survival/death decisions and neuronal differentiation. TGF- β s fall into several subfamilies including the TGF- β s 1, 2, and 3, the bone morphogenetic proteins (BMPs), the growth/differentiation factors (GDFs), activins, and inhibins. TGF- β signal through heteromeric complexes of type II and type I serine-threonine kinase receptors ([►receptor serine/threonine kinases](#)), which activate the downstream Smad signal transduction pathway. Three classes of Smads have been defined: receptor-regulated Smads (R-Smads), common-mediator Smads (co-Smads) and inhibitory Smads (I-Smads). TGF- β binding results in the phosphorylation of Smad2 (R-Smad), its dissociation from the receptor and assembly of heteromeric complexes with Smad4 (co-Smad), which finally translocates to the nucleus where it modulates gene expression. Additionally, there are also SMAD-independent signaling pathways available, involving TAK1/JNK and p38, Ras-Erk, RhoA-p160ROCK or PP2A-S6K pathways.

New Factors

Conserved dopamine neurotrophic factor (CDNF) is a newly discovered growth factor with a high specificity and activity to promote survival of dopaminergic neurons in vitro and in vivo. CDNF is homologous to an astrocyte-derived growth factor, MANF-1.

Clinical Use (Including Side Effects)

Over the past 15 years, neurotrophic factors have generated considerable excitement because of their therapeutic potential for a wide variety of currently incurable degenerative neurological disorders. Attempts to replicate the success of animal studies in demonstrating the therapeutic efficacy in clinical trials has been less successful. CNTF, BDNFs and IGF-I have been tested in clinical trials for the treatment of [►amyotrophic lateral sclerosis](#), NGF in clinical trials for [►peripheral neuropathies](#) and GDNF in trials for [►Parkinson's disease](#) [2,4,5]. However, many issues with regard to technical and pharmacological parameters, such as drug delivery to the site of action, and mode of application, remain to be solved before a final judgement on the use of neurotrophic factor therapy can be drawn.

►Growth Factors

►Hematopoietic Growth Factors

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Neurotrophin

Neurotrophins are peptides or protein molecules that regulate both cell survival and cell death of specific neuronal phenotypes and thus serve to influence development, maintenance, function, and plasticity of the nervous system.

►Neurodegeneration

►Neurotrophic Factors

Neutral Antagonist

A neutral antagonist binds equally to both active and inactive states of a G-protein-coupled receptor, regardless of activation state, and therefore blocks the actions of agonists and inverse agonists alike.

►Drug-Receptor Interaction

Neutral Endopeptidase

Neutral endopeptidase (NEP, neprilysin) is an enzyme that preferentially catalyzes cleavage at the amino group of hydrophobic residues of the B-chain of insulin

as well as opioid peptides and other biologically active peptides. The enzyme is inhibited primarily by EDTA, phosphoramidon and thiorphan and is reactivated by zinc. NEP is identical to common acute lymphoblastic leukemia antigen (CALLA), a marker protein of human acute lymphocytic leukaemia.

► Endothelins

Neutropenia

Neutropenia is a drop in the number of circulating leukocytes, especially neutrophils. It can be induced by a variety of drugs. Treatment with cytotoxic antineoplastic drugs usually results in severe neutropenia, which can be treated with colony-stimulating factors (G-CSF, GM-CSF).

Neutrophils

► Inflammation

NF-κB

► Nuclear Factor-kappaB

NF-IL6

NF-IL6 is a nuclear factor for interleukin-6, a transcription factor which is activated by IL-6 and other cytokines and stimulates stress protein gene expression.

► Cytokines

NFAT Family of Transcription Factors

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Synonyms

Nuclear factor of activated T cells cytosolic NFATc

Definition

Originally described in the immune system, NFAT proteins comprise a family of transcriptional factors that play key roles in many cellular processes that control not only immune responses but also the development, regulation, and differentiation of many other tissues. Activation of NFAT proteins results in the expression of specific sets of genes that regulate multiple cell functions [1, 2].

Structure and Members

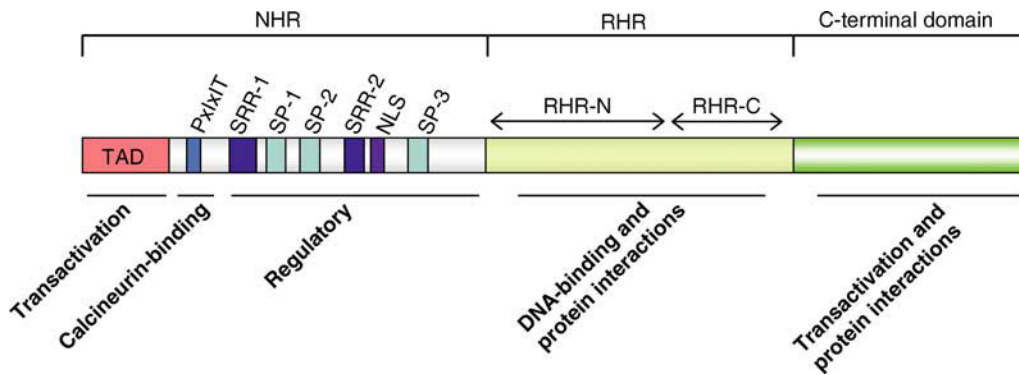
The NFAT family encompasses five different proteins that form part of the extended ►Rel/NF-κB family. The five NFAT members are: NFAT1 (also known as NFATc2 or NFATp), NFAT2 (NFATc1 or NFATc), NFAT3 (NFATc4), NFAT4 (NFATc3 or NFATx), and NFAT5 (TonEBP or OREBP). NFAT5 is the only NFAT member that is not regulated by calcium. This protein also lacks the conserved regulatory domain present in the other NFATs and its activation occurs in response to osmotic stress. This chapter will focus exclusively on the four calcium-regulated NFAT family members.

NFAT proteins contain three distinct domains: the NFAT homology region (NHR), the Rel-homology region (RHR), and the C-terminal domain (Fig. 1). The NHR is conserved in all NFATs and contains a strong transactivation domain (TAD) and the regulatory domain, which is essential for the calcium-mediated regulation of NFAT. The regulatory domain contains several phosphorylated serines, the docking sites for ►calcineurin (Cn) and the NFAT kinases, and the sequences that control the shuttling of NFAT between the nucleus and the cytoplasm. The RHR is highly conserved within all NFAT proteins and shares structural homology with the Rel domain of the NF-κB family of transcription factors. It contains the DNA-binding domain, which is also involved in protein-protein interactions. In the C-terminal resides a TAD, although its function has not been fully characterized yet [2].

Basic Mechanisms

Regulation of NFAT Activation

The activation of NFAT is regulated by calcium. In resting cells, NFAT proteins localize in the cytoplasm



NFAT Family of Transcription Factors. Figure 1 Schematic structure of NFAT proteins. The three main domains of NFAT are shown: the NFAT homology region (NHR), the Rel-homology region (RHR), and the C-terminal domain. The NHR contains the Calcineurin docking site (PxIxIT), the serine-rich regions (SRR), and SPXX-repeat motifs (SPs), which contain the phosphoserine residues that are removed by Calcineurin, the nuclear localization signal (NLS), and the transactivation domain (TAD). The RHR has two different domains, the N-terminal (RHR-N) and the C-terminal (RHR-C), which have specific functions in ensuring DNA contacts and protein–protein interactions. The C-terminal domain also contains an activation domain.

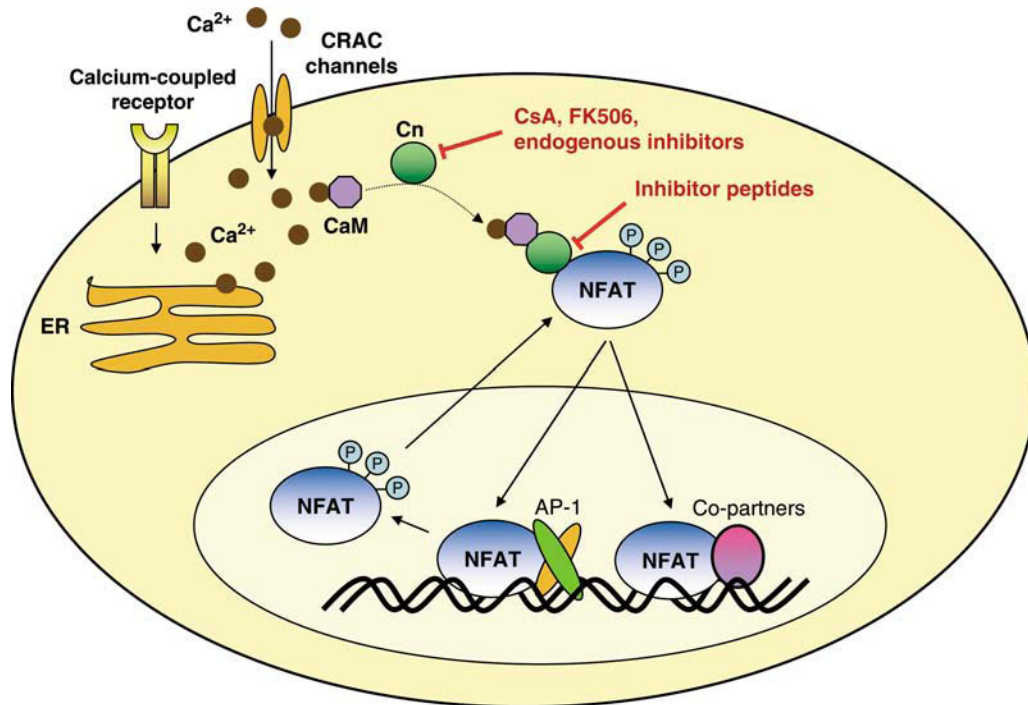
and are highly phosphorylated. Engagement of calcium signaling-coupled receptors causes an increase of intracellular calcium that activates Calcineurin, a **►calmodulin** (CaM)-dependent phosphatase. Activated Calcineurin dephosphorylates NFAT inducing its translocation into the nucleus. In the absence of sustained calcium signaling, NFAT is rephosphorylated by nuclear kinases and exported back into the cytoplasm (Fig. 2). Phosphorylation of NFAT proteins occurs in the serine-rich motifs localized in the NHR (see Fig. 1) and controls both cytoplasmic retention and nuclear export. Several cytosolic and nuclear kinases are required to control activation of all calcium-regulated NFAT proteins, including casein kinase 1 and the glycogen synthase kinase 3. Other kinases have been shown to be specific for a particular NFAT family member, like the mitogen-activated protein kinases (MAPKs) p38 and JNK, which specifically phosphorylate NFAT1 and NFAT2, respectively [1, 2].

The NHR contains also the conserved Calcineurin docking site, PxIxIT, required for the physical interaction of NFAT and Calcineurin. Dephosphorylation of at least 13 serines residues in the NHR induces a conformational change that exposes the **►nuclear localization sequences** (NLS), allowing the nuclear translocation of NFAT. Rephosphorylation of these residues unmasks the nuclear export sequences that direct transport back to the cytoplasm. Engagement of receptors such as the antigen receptors in T and B cells is coupled to phospholipase C activation and subsequent production of inositol triphosphate. Increased levels of inositol triphosphate lead to the initial release of intracellular stores of calcium. This early increase of calcium induces opening of the plasma membrane calcium-released-activated-calcium **►(CRAC) channels**,

which allow entry of extracellular calcium guaranteeing sustained levels of calcium in the cytosol. This sustained calcium mobilization is required to maintain Calcineurin enzymatic activity. The calcium binding protein CaM is activated and binds Calcineurin, displacing its autoinhibitory domain. Activated Calcineurin is then capable of dephosphorylating and activating NFAT. Although the regulation of NFAT proteins is mostly dependent on the intracellular calcium concentrations and on the enzymatic activity of Calcineurin and NFAT kinases, other mechanisms of regulation can also play important roles. For example, the specific NFAT2/ α A isoform promoter contains a NFAT binding site that allows a positive transcriptional autoregulation of this isoform. Similarly, signaling pathways that control the activation of transcription factors cooperating with NFAT also play crucial roles in the overall modulation of NFAT activity.

Mechanisms of Action

Activated NFAT proteins bind DNA and interact with other transcription factors to positively or negatively regulate the transcription of many genes. The RHR contains the DNA binding domain, which is responsible for the interaction of NFAT proteins with specific DNA sequences. It is also mainly through this domain that NFAT can interact with other transcriptional factors to form higher-order transcriptional complexes. The RHR can adopt different structural conformations on DNA binding sites, which allows NFAT to interact with specific transcriptional partners on each NFAT-regulated gene promoters or enhancers. Cooperative interactions with members of the **►activator protein-1** (AP-1) family of transcription factors regulate most NFAT-dependent gene transcription induced in activated immune cells. Several other transcriptional partners have been identified for



NFAT Family of Transcription Factors. Figure 2 Regulation of NFAT activation. Extracellular stimuli activate receptors such as the antigen receptor in T cells that lead to the release of calcium from the intracellular stores in the endoplasmic reticulum (ER) and subsequent opening of the calcium-released-activated-calcium (CRAC) channels. Calcium binds and activates calmodulin (CaM), which in turn binds and activates Calcineurin (Cn). Calcineurin dephosphorylates NFAT inducing its nuclear translocation. Cooperation of NFAT and other transcription factors (e.g., AP-1 proteins) in the nucleus promotes gene transcription. NFAT kinases rephosphorylate NFAT, which induces export back to the cytoplasm and the termination of NFAT activation. NFAT activity may be pharmacologically inhibited by blocking Calcineurin enzymatic activity with cyclosporine A or FK506, or interrupting the interaction of Calcineurin and NFAT with inhibitor peptides [3].

NFAT proteins, for which functional synergy and/or physical protein/protein interaction with an NFAT protein have been described. Positive synergy has been shown between NFAT and Maf, GATA-proteins, MEF2, IRF4, C/EBP, Oct, Egr transcription factors, and PPAR γ ; while interactions with ICR and p21^{snf} negatively regulate NFAT activity. The nature of these complexes is responsible for the activation or suppression of specific sets of genes in multiple tissues in response to different stimuli [4, 5].

NFAT Functions

NFAT was first described in cells of the immune system, but these transcription factors are widely expressed and their function is crucial in the regulation, development, and differentiation of many nonimmune tissues.

Immune System

NFAT was initially identified in activated T cells as a DNA binding activity required for IL-2 expression, a cytokine that plays a key role in T cell activation and survival. Subsequent studies revealed the involvement

of NFAT in the expression of many other cytokines including, among others, IL-3, IL-4, IL-5, IL-10, IL-13, GM-CSF, TNF, and INF γ , as well as several cell surface receptors, such as CD40L, FasL, and IL2-R α , required for proper regulation of immune responses. Activation of T cells by engagement of the **T cell receptor (TCR)** and costimulatory molecules (i.e., CD28) results in the increase of free cytosolic calcium and in the activation of the RAS-MAPK and PKC signaling pathways. Engagement of these signaling pathways induces the activation of members of the NFAT and AP-1 families of transcription factors. In the nucleus, NFAT and AP-1 proteins form cooperative complexes to induce the expression of many activation-induced genes. As described above, other transcriptional partners also play important roles modulating NFAT-mediated gene expression in T cells [4].

In the absence of costimulation, T cells that receive signaling through the TCR become **anergic** and unresponsive to new antigen stimulation. The anergic state in T cells results from activation of NFAT in the absence of AP-1. In this scenario, a specific set of genes is

upregulated. The proteins encoded by these genes are responsible for blocking TCR signaling in anergic T cells.

Besides being a key regulator of mature T cell function, NFAT is also required in T cell development and T-helper cell differentiation. Studies performed on NFAT or Calcineurin-deficient mice have confirmed that NFAT signaling is involved in thymocyte development. NFAT proteins regulate thymocyte proliferation and survival as well as the development of immature CD4⁺ CD8⁺ thymocytes into mature CD4⁺ or CD8⁺ T cells. Cooperation between NFAT proteins and STAT factors is involved in the establishment of the epigenetic changes that determine activation of distinct cytokine genes in specific T helper cell subpopulations. Cooperation with lineage-specific transcription factors, like GATA-3 in T helper 2 cells, is also required to activate the expression of those genes.

The role of NFAT in the immune system is not restricted to T cells. NFAT proteins are also expressed in other cells of the immune system, such as B cells, NK cells, and mast cells, where they have been shown to regulate the expression of cytokines, cell surface receptors, and immunoglobulins [4, 5].

Musculoskeletal System

NFAT proteins are expressed in skeletal, cardiac, and smooth muscle and play important roles in the regulation of the development and differentiation of these tissues. In skeletal muscle, NFAT isoforms are expressed at different stages of development and regulate progression from early muscle cell precursors to mature myocytes. NFAT proteins have also been shown to control the expression of the myosin heavy chain and positively regulate muscle growth [1, 2].

Analysis of animals deficient in NFAT1 has shown that NFAT negatively regulates cartilage growth. Bone remodeling is also regulated by calcium/NFAT signaling. NFAT controls osteoclast and osteoblast differentiation, coupling bone formation and resorption during skeletal development and repair. In osteoclasts, increased intracellular calcium promoted by RANKL signaling activates NFAT2, which promotes the expression of several osteoclast-specific genes [2].

Cardiovascular System

NFAT2 plays a key role in the development of the embryo's heart. In the precursor cells, there is a temporal and spatial specific expression of NFAT2, which directs the formation of the valves and the septum in the heart. In the adult heart, NFAT proteins also cooperate with transcription factors of the GATA and MEF2 families to regulate cardiac muscle hypertrophic responses.

Calcineurin signaling and NFAT also play an important role in the regulation of vascular development, balancing activation and inhibition of angiogenesis. NFAT3 and

NFAT4 are expressed in perivascular mesenchymal cells, which are implicated in the assembly of blood vessels during embryogenesis. The lack of NFAT signaling results in a disorganized and inappropriate growth of developing vessels. In endothelial cells, Vascular Endothelial Growth Factor activates NFAT-dependent transcription of endothelial genes like Cyclooxygenase-2 and promotes endothelial cell migration.

Other Systems

NFAT proteins have also been shown to modulate many other cellular functions. For instance, they regulate neuronal axon growth and are also involved in insulin homeostasis, by controlling beta cell growth and regulating insulin-signaling pathways and adipogenesis. Given that one or more NFAT family members are expressed in almost any tissue, and that recent data have implicated NFAT proteins in the control of essential processes in new cell types, it is becoming increasingly evident that the calcium/Calcineurin/NFAT axis constitutes a crucial signaling pathway for the regulation of the development and function of many different tissues.

Pharmacological Relevance

The development of drugs that block Calcineurin phosphatase activity has allowed successful prevention of graft rejection. Due to the key role that NFAT has in T-cell activation, these inhibitors behave as potent immunosuppressors. Nevertheless, the important roles that NFAT proteins have in other tissues suggest that exploring the use of similar drugs in other pathological contexts may be clinically and therapeutically relevant.

Classical Calcineurin Inhibitors

Cyclosporin A and FK506 are the most commonly used immunosuppressant drugs and their clinical use has transformed the field of organ transplantation. Both drugs have different structures but share a similar mechanism of action: inhibiting Calcineurin enzymatic activity. For successful inhibition, Cyclosporin A and FK506 must form a complex with their intracellular receptors, cyclophilin A and the FK506 binding protein FKBP12, respectively. These complexes bind then to sites located in the region of the Calcineurin A subunit that interacts with Calcineurin B, and inhibit the phosphatase activity required for NFAT activation. The crucial role that Calcineurin and NFAT signaling play in the activation of T and B cells and in the control of many of the genes that regulate immune responses (i.e., cytokines, cytokine receptors, and costimulatory molecules) underlies the effectiveness and potency of these drugs. So far Cyclosporine A and FK506 are the most potent immunosuppressive drugs in clinical use, despite their severe side effects, specially their nephrotoxicity. To

diminish the toxicity of these immunosuppressants, synthetic inhibitors are being designed with similar inhibitory activity on Calcineurin but with reduced renal toxicity [3].

Other Inhibitors

The search for new inhibitors of NFAT with higher specificity and lower toxicity has led to the design of specific peptides that are able to block the NFAT/Calcineurin interaction. The docking site of Calcineurin in NFAT has been used to design small peptides that specifically prevent the binding of Calcineurin to NFAT proteins. Analysis of a combinatorial library of P_xI_xIT-based peptides has produced a high affinity peptide that, in experimental in vitro and animal models, blocks NFAT binding to Calcineurin, inhibits NFAT-dependent transcription and prolongs allograft acceptance in mice. Therapeutic use of peptide inhibitors may be hindered by problems associated with delivery and product stability. Small organic molecules, with almost limitless structural possibilities, also offer an exciting possibility to design new inhibitors with improved specificity, stability, delivery, and tissue distribution. Being able to selectively inhibit NFAT makes the future development of these drugs an attractive alternative to the classical inhibitors of Calcineurin activity, as they can offer similar or even better therapeutic effectiveness with fewer secondary effects [2].

► Immune Defense

► Immunosuppressive Agents

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NGF

► Nerve Growth Factor

► Neurotrophic Factors

NHE

► Na⁺/H⁺ Exchangers

Niacin

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Definition

Niacin (Fig. 1) is a collective name for all vitamers having the biological activity associated with nicotinamide (= pyridine-3-carboxamide), including nicotinic acid (= pyridine-3-carboxylic acid) and a variety of pyridine nucleotide structures.

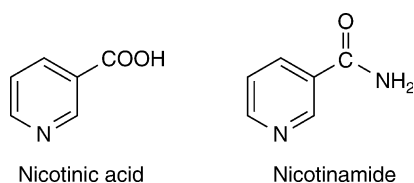
Nicotinamide and nicotinic acid are both white crystalline substances. Their aqueous solution has a maximal UV absorbance at 263 nm. Both vitamers have the same biological activity as they can be converted into each other. Figure 2 shows the structure of the coenzyme forms NAD⁺ and NADP⁺.

Most foods of animal origin contain nicotinamide in the coenzyme form (high bioavailability). Liver and meat are particularly rich in highly bioavailable niacin. Most of the niacin in plants, however, occurs as nicotinic acid in overall lower concentrations and with a lower bioavailability. The major portion of niacin in cereals is found in the outer layer and its bioavailability is as low as 30% because it is bound to protein (niacytin). If the diet contains a surplus of L-tryptophan (Trp), e.g., more than is necessary for protein synthesis, the liver can synthesize NAD from Trp. Niacin requirements are therefore declared as niacin equivalents (1 NE = 1 mg niacin = 60 mg Trp).

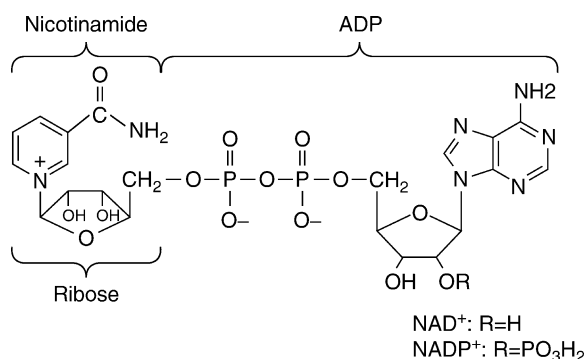
Mechanism of Action

NAD⁺ and NADP⁺ are coenzymes of dehydrogenases. NADH and NADPH are intermediate carriers of both hydrogen and electrons. Most NAD-dependent enzymes are located in the mitochondria and deliver H₂ to the respiratory chain whereas NADP-dependent enzymes take part in cytosolic syntheses (reductive biosyntheses).

NAD⁺ possesses some non-redox functions as well. The glycosidic linkage between nicotinamide and ribose is a high-energy bond. The energy provided by



Niacin. Figure 1 Structure of nicotinic acid and nicotinamide.



Niacin. Figure 2 Structure of the coenzymes NAD^+ (nicotinamide-adenine dinucleotid) and NADP^+ (nicotinamide-adenine dinucleotid phosphate).

breaking this bond allows the addition of ADP-ribose to a variety of nucleophilic acceptors.

Researchers found that NAD serves as a substrate in poly(ADP-ribose) synthesis, a reaction important for DNA repair processes. In addition, it takes part in mono (ADP-ribosyl)ation reactions that are involved in endogenous regulation of many aspects of signal transduction and membrane trafficking in eukaryotic cells.

NADP can be converted to nicotinic acid adenine dinucleotide phosphate (NAADP), which has distinct functions in the regulation of intracellular calcium stores. The studies of these new roles of NAD(P) in metabolism are in their early stages, but they might soon help to better understand and explain the symptoms of niacin deficiency (►pellagra) [1].

Clinical Use (Including Side Effects)

Nicotinic acid is used in the treatment of hyperlipidemia. It causes various changes in lipid and lipoprotein metabolism when administered in high doses (up to 5 g/d):

The maximum changes achieved in a study were –20% total serum cholesterol, –40% serum triglycerides and +15% HDL-cholesterol [2]. However, there are considerable short- and long-term side-effects. The treatment should therefore be monitored by a doctor.

Nicotinamide potentiates the cytotoxic effects of chemotherapy and radiation treatment against tumor cells. This effect is probably attributable to increased

blood flow and oxygenation of the tumor tissue. Furthermore, insulin-dependent ►diabetes mellitus might be prevented by the administration of high doses of nicotinamide (about 3 g/d) according to recent studies. The mechanisms have yet so far not been fully elucidated and potential side-effects not completely explored [2].

- Folic Acid
- Biotin
- Pantothenic Acid
- Vitamin B1
- Vitamin B2
- Vitamin B6
- Vitamin B12
- Vitamin C

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Nicotinic Acetylcholine Receptor

- nAChR
- Nicotinic Receptors
- Table appendix: Receptor Proteins
- Non-selective Cation Channels

Nicotinic Receptors

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Synonyms

Nicotinic acetylcholine receptor

Definition

The nicotinic receptor (nAChR) comprises a family of receptor subtypes that respond to the neurotransmitter acetylcholine (ACh) and the tobacco alkaloid nicotine.

nAChRs are found in:

1. Skeletal muscle (muscle nAChR, comprised of $\alpha 1, \beta 1, \gamma$ or ϵ , and δ subunits, also present in electric tissues of *Torpedo* and *Electrophorus*)
2. Sympathetic, parasympathetic and sensory neurones, chromaffin, neuroblastoma and PC12 cells (ganglionic nAChR, typically comprised of $\alpha 3, \alpha 5, \beta 2, \beta 4$ or $\alpha 7$ subunits)
3. CNS neurones (in mammals: heteromeric nAChR comprised of α/β combinations from $\alpha 2, \alpha 3, \alpha 4, \alpha 5, \alpha 6, \beta 2, \beta 3, \beta 4$ subunits, or homomeric nAChR comprised of $\alpha 7$ subunits only)
4. Non-neuronal cells (including astrocytes, mechanosensory hair cells, macrophages, keratinocytes, endothelial cells of the vascular system, muscle cells, lymphocytes, intestinal epithelial cells and various cell-types of the lungs)

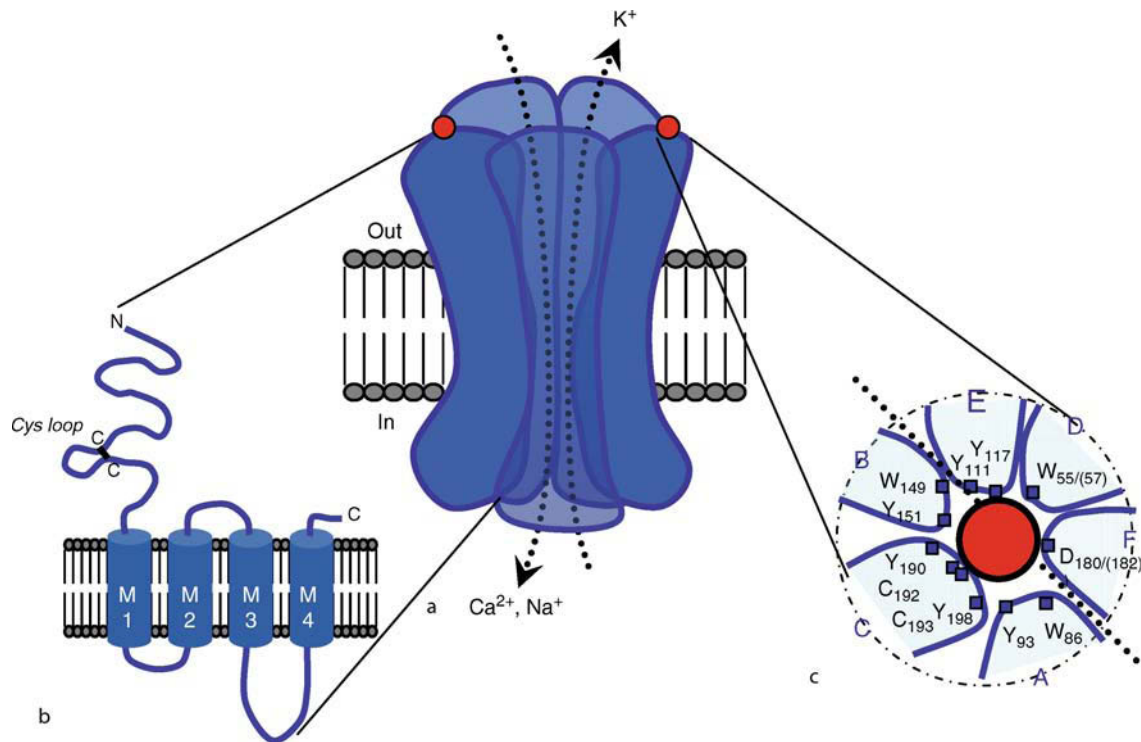
5. Invertebrate nervous systems (invertebrate nAChR)

The subtypes of nAChR that are not found in skeletal muscle are collectively referred to as 'neuronal nAChRs'.

The muscle nAChR is the prototype of the cys-loop family of ligand-gated ion channels (LGIC) that also include GABA_A, GABA_C, glycine, 5HT₃ receptors, and invertebrate glutamate-, histamine-, ACh-, and 5HT-gated chloride channels.

Basic Characteristics

The nAChR is comprised of five subunits, each of which spans the lipid bilayer to create a water-filled pore or channel (Fig. 1a). Each subunit consists of four transmembrane segments, the second transmembrane segment (M2) lines the ion channel (Fig. 1b). The extracellular N-terminal domain of every subunit



Nicotinic Receptors. Figure 1 (a) Schematic representation of a nAChR. Five subunits span the lipid bilayer to create a water-filled central channel that is permeable to cations when opened in response to agonist binding. Binding of two agonist molecules (represented by red spheres) is required for channel opening. (b) Structure of a single subunit. Each subunit has a large extracellular domain containing the 'cys loop' and traverses the membrane 4 times; the second transmembrane spans 3 and 4 that contains putative phosphorylation sites; this region is likely to be important for regulation, trafficking and interaction with cytoskeletal and scaffolding proteins. (c) The agonist-binding site. Agonist (red sphere) binds to a site at the interface between two subunits. The primary (α) subunit contributes amino acids in three loops (A,B,C) to bind the agonist, supported by additional residues in three loops (D,E,F) from the complimentary face of the adjacent subunit (γ, ϵ or δ in muscle nAChR, α or β in neuronal nAChR). The amino acids indicated by their single letter codes are numbered according to the nAChR from *Torpedo* electric tissue. This source has provided large quantities of muscle-like nAChR for analysis.

contains a 'cys loop' that is the signature sequence of the LGIC family: two cysteine residues, separated by 13 amino acids form a disulphide bond to create a loop. The binding site for agonists, including ACh and nicotine, occurs at the interface between adjacent subunits, in the extracellular part of the receptor created by the N-terminal domains of its subunits. The primary binding site is found on α subunits that possess a pair of adjacent (vicinal) cysteine residues and a number of key hydrophobic residues organised in three loop structures (A,B,C) that coordinate the agonist (Fig. 1c). The complimentary subunit (γ or ϵ and δ in muscle nAChR; α or β in neuronal nAChR) also contribute three loops (D,E,F) containing hydrophobic residues. In muscle and $\alpha\beta$ heteromeric neuronal nAChR there are two binding sites and both must be occupied by agonist in order to open the ion channel.

In the resting (closed) state, the ion channel is occluded by a 'hydrophobic girdle' that constitutes a barrier to ion permeation. Agonist binding in the extracellular domain promotes a conformational change that results in a rotational movement of the M2 helices lining the pore, widening the pore by ~ 3 Å. This results in an influx of Na^+ and Ca^{2+} (the relative permeability of these two cations depends on nAChR subtype), and an efflux of K^+ under normal physiological conditions. Despite the presence of agonist, the nAChR channel closes within seconds to minutes, to enter a ►desensitised state. In this condition, the nAChR is refractory to activation. Multiple desensitised states have been proposed to exist.

At the muscle endplate, nAChR activation produces ►depolarisation that triggers muscle contraction. In sympathetic ganglia, postsynaptic nAChRs also mediate synaptic transmission, resulting in the depolarisation of postganglionic neurones and the increased likelihood of generating an action potential. In the vertebrate CNS this postsynaptic function is less common. nAChR are more often found at presynaptic and extrasynaptic locations on brain neurones and exert a modulatory influence on brain function. This is achieved by alteration in excitability or increases in intracellular Ca^{2+} . Thus presynaptic nAChR stimulate the release of various neurotransmitters in different brain regions; somatodendritic nAChR can promote long-term changes by altering gene expression.

The $\alpha 7$ homomeric nAChR has attracted interest because of its high relative permeability to Ca^{2+} (comparable to that of the NMDA receptor). Its association with glutamate terminals supports a contribution to ►long-term potentiation (LTP) through presynaptic facilitation of glutamate release. Postsynaptically, $\alpha 7$ nAChR can activate several cell signalling pathways, leading to activation of ERK, CREB and ►immediate early genes such as cFos.

Drugs

Nature has created a diverse array of plant and animal toxins that act at mammalian muscle and ganglionic nAChRs or invertebrate nAChRs because the critical physiological functions of these receptors make them prime targets for defensive or predatory strategies. More recently, the perceived validity of neuronal nAChR as therapeutic targets has prompted the generation of new synthetic ligands. Examples are listed in Table 1.

Agonists activate nAChR by binding to the agonist-binding site (Fig. 1). They can remain bound (often with higher affinity) when the nAChR enters the desensitised state.

Competitive Antagonists interact reversibly with the nAChR at, or close to, the agonist-binding site, stabilising the receptor in a conformation with the channel closed and preventing agonists from accessing their binding site. Inhibition by reversible competitive antagonists is surmountable with increasing agonist concentration, shifting the concentration response relationship to the right.

Non-competitive antagonists do not compete for binding to the agonist-binding sites, but interact with distinct sites that modify nAChR function. Their inhibition is not surmountable with increasing agonist concentration. Some non-competitive antagonists are 'channel blockers' that act either by blocking the entrance to the nAChR channel or entering the lumen of the channel. The action of such drugs can be 'state-dependent', requiring prior activation of the nAChR to allow access to the channel. Many compounds that have other primary targets can also act as non-competitive antagonists of nAChR. Examples include antagonists of voltage operated Ca^{2+} channels, NMDA channel blockers, antidepressants, steroids, β amyloid peptide.

Positive allosteric modulators potentiate responses to nicotinic agonists by acting at a site distinct from the agonist-binding site. Such effects are well established for other receptor classes (e.g. benzodiazepines acting at GABA_A receptors), but have only relatively recently been characterised for neuronal nAChR. The acetylcholinesterase inhibitors galanthamine and physostigmine, the neurotransmitter 5HT and the opiate codeine can enhance responses to agonists, without discriminating between nAChR subtypes. The 5HT metabolite 5-hydroxyindole and a novel synthetic molecule PNU-120596 are selective modulators of $\alpha 7$ nAChR.

Therapeutic or Agrochemical Applications

Historically ganglionic nAChR have been targets for treating hypertension. The discovery of a large family of nAChR subtypes in the CNS, coupled with observations that nicotine has anti-nociceptive, neuroprotective and cognitive effects, has led to the recognition that neuronal nAChR are potential targets

Nicotinic Receptors. Table 1 Some drugs that act at nAChRs

	Drug	Source	Specificity
Agonists	ACh	Endogenous agonist	–
	(±)-Anatoxin-a	<i>Anabaena flos aquae</i> (Blue green algae)	–
	Carbamylcholine	Synthetic carbamate analogue of ACh	Muscarinic > nicotinic
	Choline	Endogenous precursor and $\alpha 7$ agonist	$\alpha 7$ nAChR
	(–)-Cytisine	<i>Fabaceae</i> genera	–($\alpha 4\beta 2$ partial agonist)
	Dimethylphenyl piperazinium (DMPP)	Synthetic	Ganglionic
	(±)-Epibatidine	<i>Epipedobates tricolour</i> (South American frog)	–
	Imidacloprid	Synthetic chloro-nicotinyl insecticide	Insect CNS nAChR
	5-Iodo-A-85380	Synthetic	$\beta 2$ -containing > others
	Levamisole	Synthetic imidazothiazole	nematode muscle nAChR
	Lobeline	<i>Lobelia inflata</i> ,	–(also non-specific actions)
	(–)Nicotine	<i>Nicotiana tabaccum</i>	–
	<i>Trans</i> -metanicotine (TC-2403)	Synthetic	$\beta 2$ -containing > others
	Varenicline	Synthetic	$\alpha 4\beta 2$ partial agonist
Competitive antagonists	α -Bugarotoxin	<i>Bungarus multicinctus</i> (banded krait)	Muscle/ $\alpha 7, \alpha 8, \alpha 9, \alpha 10$ /some invertebrate nAChRs
	α -Conotoxin	<i>Conus</i> sp.	Various/subtype-specific
	Decamethonium	Synthetic	Muscle > ganglionic
	Dihydrobetaerythroidine (DH β E)	<i>Erythrina</i> sp.	$\beta 2 > \beta 4$
	d-Tubocurarine	<i>Strychnos toxifera</i> ; <i>Chondrodendron tomentosum</i>	–
	Methyllycaconitine (MLA)	<i>Delphinium brownii</i>	$\alpha 7, \alpha 8, \alpha 9, \alpha 10$ /some invertebrate nAChRs
Non-competitive antagonists	Chlorisondamine	Synthetic	Ganglionic (long-lasting blockade of CNS nAChRs)
	Hexamethonium	Synthetic	Ganglionic
	Histrionicotoxin	<i>Dendrobates histrionicus</i>	–
	Mecamylamine	Synthetic	–

for treating a wide range of disorders. These include Alzheimer's and Parkinson's diseases, schizophrenia, attention-deficit hyperactivity disorder (ADHD), pain. nAChRs in peripheral cells are of interest in treating inflammation in different scenarios including ulcerative colitis and wound healing. Nicotine, cytisine (Tabex) and varenicline (Chantix, Champix) are in use for smoking cessation.

Imidacloprid is a widely used neonicotinoid insecticide that kills pests by targeting their central nAChRs. Levamisole is used to kill nematodes by acting on nAChRs in the worm's muscles.

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Nigrostriatal Tract/Pathway

The nigrostriatal tract is one of the four main dopaminergic pathways in the central nervous system. About 75% of the dopamine in the brain occurs in the nigrostriatal pathway with its cell bodies in the substantia nigra, whose axons project in the corpus striatum. Degeneration of the dopaminergic neurons in the nigrostriatal system results in Parkinsons disease.

► Dopamine System

Nitrates

A family of clinically used compounds that release NO and relax most smooth muscles.

► Smooth Muscle Tone Regulation
► Guanylyl Cyclases

Nitroergic Nerve

The nerve whose transmitter function depends on the release of NO. Nonadrenergic, noncholinergic inhibitory responses of smooth muscle are associated mainly with activation of nitroergic nerves.

► Nitric Oxide

Nitroergic Transmission

Nitroergic transmission is synaptic transmission by nitric oxide. In contrast to other transmitters, NO is not preformed and stored in synaptic vesicles. When an

action potential arrives at a nitroergic terminal, the Ca^{2+} entered through the presynaptic Ca^{2+} channels activates neuronal NO synthase and NO is then produced ad hoc from arginine. It is released not by exocytosis but by diffusion through the plasma membrane. In further contrast to other transmitters, the postsynaptic receptor is not a membrane protein but the cytosolic enzyme soluble guanylyl cyclase, which catalyzes formation of the second messenger cyclic GMP. Finally, NO is not inactivated by enzymatic degradation or cellular uptake but by spontaneous oxidation. NO is a transmitter in the enteric nervous system and in some blood vessels where it causes vasodilation.

► Synaptic Transmission
► Nitric Oxide

Nitric Oxide

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Synonyms
NO

Definition

In contemporary societies replete with various industries and automobiles, NO_x (NO, NO_2 , and NO_3) has been recognized to be one of the important factors responsible for air pollution. Only two decades ago, NO was found to be an essential molecule that regulates cellular/molecular functions in mammals. NO is also enzymatically synthesized in nonmammals, invertebrates, and yeasts. Therefore, the origin of NO may date back to the birth of life arising from single cell organisms living around 3-billion years ago.

Furchgott and Zawadzki [1] first discovered that endothelial cells release a substance(s) responsible for the relaxation of vascular smooth muscle by acetylcholine; this substance was named “► endothelium-derived relaxing factor (EDRF).” This epoch-making discovery answers the question raised for nearly one hundred years by pharmacologists about why vascular smooth muscle is relaxed by acetylcholine, which however elicits contraction of the other smooth muscles. Because of its instability, the true chemical nature of EDRF was not easily identified. Several years later, several research groups independently found that the biological activities and biochemical properties of EDRF were identical

to those of NO. In the early 1990s, NO synthesizing enzymes and processes were elucidated, enabling researches to develop specific inhibitors for the enzymes involved. This was the dawn of investigations on gas physiology/pharmacology. Discovery of EDRF or endothelial NO highlighted the importance of endothelial cells that were reevaluated for additional physiological significance. Functional importance of NO initially found as an EDRF in blood vessels rapidly expanded as novel findings were obtained about its diverse functions in the whole body [2], including the central and peripheral nervous systems, autonomically innervated organs and tissues, endocrine/exocrine systems, cell differentiation and development, fertility, and immunological responses. Because of their outstanding discoveries of NO and its important physiological roles and pathophysiological implications, Drs. R. F. Furchgott, L. J. Ignarro, and F. Murad, all superb pharmacologists, were honored with the Nobel Prize (Physiology section) in 1998.

Basic Mechanisms

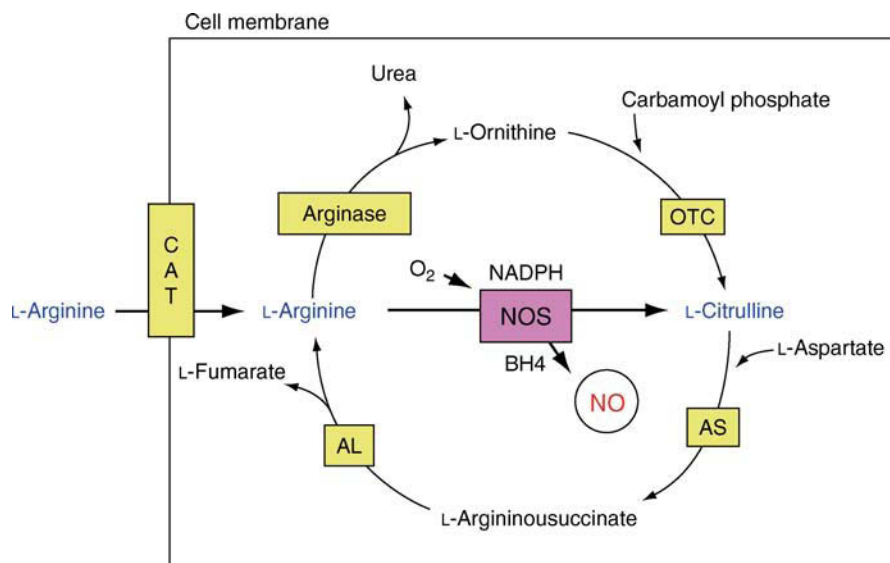
Synthesis and Degradation of NO

Three isoforms of NO synthesizing enzymes (►nitric oxide synthase (NOS)) were isolated, purified, and cloned: neuronal NO synthase (►neuronal nitric oxide synthase (nNOS) or isoform (I), immunological or inducible NOS (►inducible (immunological) nitric oxide synthase (iNOS) or isoform (II), and endothelial NOS (►endothelial nitric oxide synthase (eNOS) or isoform

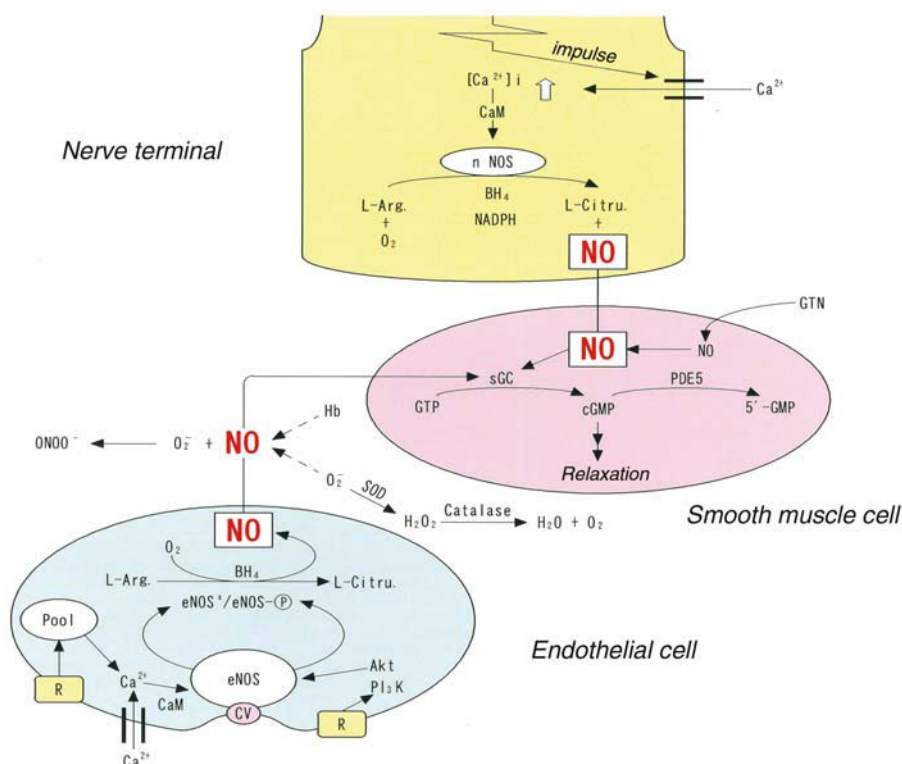
(III). The nNOS and eNOS are constitutively expressed in the central and peripheral nerves and vascular endothelial cells, respectively, and require Ca^{2+} and calmodulin in the presence of tetrahydrobiopterin (BH_4), heme, NADPH, FAD, and FMN for activation. iNOS is not constitutively expressed but is induced in macrophages by bacterial lipopolysaccharides and cytokines; Ca^{2+} is not required for activation. NO is produced when ►L-arginine is transformed to L-citrulline by catalysis of NOS in the presence of oxygen and cofactors (Fig. 1). The synthesis of NO is inhibited by L-arginine analogs, such as N^G -monomethyl-L-arginine, N^G -nitro-L-arginine, and asymmetric dimethylarginine. NO generated by enzymatic and nonenzymatic processes is rapidly inactivated by oxidation to NO_2/NO_3 . NO is scavenged by superoxide-generating compounds. Binding of NO to superoxide anions forms the bioactive compound ►peroxynitrite (Fig. 2).

Physiological and Pathophysiological Effects of NO Formed by eNOS

eNOS is present mainly in the particulate fraction of endothelial cells and binds to caveolin-1 in the caveolae, which are microdomains of the plasma membrane. Caveolin-1 inhibits eNOS activity, and this interaction is regulated by Ca^{2+} /calmodulin. The eNOS migrates intracellularly in response to increased cytosolic Ca^{2+} in the presence of calmodulin (Fig. 2) and is activated for NO production. The transmembrane influx of Ca^{2+} and the mobilization from its intracellular



Nitric Oxide. Figure 1 Scheme of the synthesis of NO from L-arginine and its regulatory factors. AS, argininosuccinate synthase; AL, argininosuccinate lyase; OTC, ornithine transcarbamylase; CAT, cationic amino acid transporter. Km values for arginases I and II approximate 1,000 times higher than those for NOS isoforms. However, once arginases are induced by cytokines, arginases and NOS compete for the utilization of substrate L-arginine.



Nitric Oxide. Figure 2 Scheme of the regulation of vascular smooth muscle function by NO released from the nitrergic nerve and endothelial cell. CaM, calmodulin; L-Arg., L-arginine; L-Citru., L-citrulline; GTN, glyceryltrinitrate (nitroglycerin); sGC, soluble guanylyl cyclase; cGMP, cyclic GMP; PDE5, phosphodiesterase-5; O_2^- , superoxide anion; $ONOO^-$, peroxynitrite; SOD, superoxide dismutase; eNOS*, activated eNOS by Ca^{2+} and calmodulin; eNOS-P, phosphorylated eNOS; Pool, Ca^{2+} stored site; PI_3K , phosphatidylinositol 3 kinase; CV, caveolin; R, receptor. In endothelial cells, eNOS is activated by two separate pathways, Ca^{2+} dependent and Ca^{2+} -independent. Dotted lines, inhibition. Similar mechanisms underlying nitrergic nerve-mediated smooth muscle relaxation are seen in the gastrointestinal tract, urinary tract, and corpus cavernosum penis. In the gastrointestinal tract, NO-mediated relaxations are mediated not only by cyclic GMP but also by hyperpolarization (inhibitory junction potential) associated with cyclic GMP-independent Ca^{2+} -activated K^+ channel opening.

stored sites are caused by stimulation of drug receptors in the endothelial cell membrane or by mechanical stresses applied to endothelial cells. Recently, novel mechanisms responsible for activation of eNOS without increased influx of Ca^{2+} have been revealed. Shear stress, insulin, or bradykinin induces the phosphorylation of Ser^{1177/1179} of eNOS through phosphatidylinositol-3 (PI_3) kinase and the downstream serine/threonine protein kinase Akt (protein kinase B) (Fig. 2), resulting in enhanced NO synthesis.

The release of NO from the endothelium is induced by various chemical substances, including acetylcholine; polypeptides such as substance P, bradykinin, and arginine vasopressin; histamine; ATP/ADP; α_2 -adrenoceptor agonists; thrombin; and Ca^{2+} ionophores. NO formed in response to mechanical stimuli like shear stress or transmural pressure plays an important role in maintaining basal blood flow. Endothelial NO causes vasodilatation, decreased

vascular resistance, lowered blood pressure, inhibitions of platelet aggregation and adhesion, inhibitions of leukocyte adhesion and transmigration, and reduced smooth muscle proliferation; and it acts to prevent atherosclerosis. These effects are mediated by cyclic GMP (►cyclic guanosine monophosphate (GMP)) synthesized in target cells by soluble guanylyl cyclase that is activated by NO (Fig. 2). Endothelial dysfunction participates in serious cardiovascular and metabolic diseases such as hypertension, angina pectoris, myocardial infarction, stroke, renal failure, glaucoma, and diabetes mellitus.

NO Formed by nNOS from ►Nitrergic Nerve

Nonadrenergic noncholinergic inhibitory responses to autonomic nerve stimulation are mainly mediated through NO synthesized by nNOS; NO plays a crucial role as a neurotransmitter from the peripheral efferent nerves, thus being called “nitrergic.” This provides a

new idea that NO, a gaseous and labile compound with a molecular weight of 30, is liberated immediately upon synthesis due to nNOS activated by Ca^{2+} intracellularly introduced into nerve terminals [3], whereas classical neurotransmitters, stable organic molecules, are synthesized and stored in synaptic vesicles and liberated by way of exocytosis when intracellular Ca^{2+} is increased. The receptive site for neurally released NO is intracellular guanylyl cyclase in target organs, which is also in contrast to drug receptors for classical transmitters that are located in cell membranes.

Nitrergic nerves (postganglionic parasympathetic) innervating the vascular wall participate in maintaining vasodilatation in cerebral arteries [3] that have scarce adrenergic vasoconstrictor innervation, and they also contribute to functionally counteract the action of adrenergic vasoconstrictor nerves in peripheral blood vessels to maintain blood flow homeostasis. Cholinergic nerves are not involved in the vasomotor response. This suggests that the peripheral vascular tone is regulated by reciprocal innervation, an adrenergic vasoconstrictor and a nitrergic vasodilator. In the digestive and urinary tracts, cholinergic excitatory and nitrergic/adrenergic inhibitory nerves modulate smooth muscle tone and motility and secretory responses. Intense nitrergic innervations in the pylorus/ileocolonic junction, sphincter of Oddi, and trigonal/urethral muscles contribute to smooth muscle relaxation, allowing the passage of digested contents, bile, and urine, respectively, through the sphincters. Nitrergic nerve activation evokes penile corpus cavernosum smooth muscle relaxation and increases intracavernous pressure. The NO-cyclic GMP pathway plays a crucial role in initiating and maintaining penile erection [4].

On the other hand, afferent nitrergic nerves control some sensory information processing, such as pain and reflex.

Physiologically muscle-derived NO regulates skeletal muscle contractility and exercise-induced glucose uptake. nNOS is located at the plasma membrane of skeletal muscle and facilitates diffusion of NO to the vasculature to regulate muscle perfusion.

NO Formed by nNOS in the Brain

In the brain, NO functions mainly as a neuromodulator. NO signaling appears to be essential for two forms of neural plasticity: long-term potentiation (LTP) in the hippocampus and long-term depression in the cerebellum. These forms of neural plasticity underlie aspects of both learning and information storage in the brain. Glutamate participates mainly in synaptic interactions, but with the help of NO, the strength of excitatory input might be nonsynaptically signaled to the surrounding monoaminergic neurons in the brain. NO formed by *N*-methyl-D-aspartate (NMDA) receptor activation diffuses to adjacent nerve terminals to

modulate neurotransmitter release. NO can also regulate secretion of hormones and neuropeptides. The NO/cyclic GMP pathway may also be involved in sleep and the circadian clock.

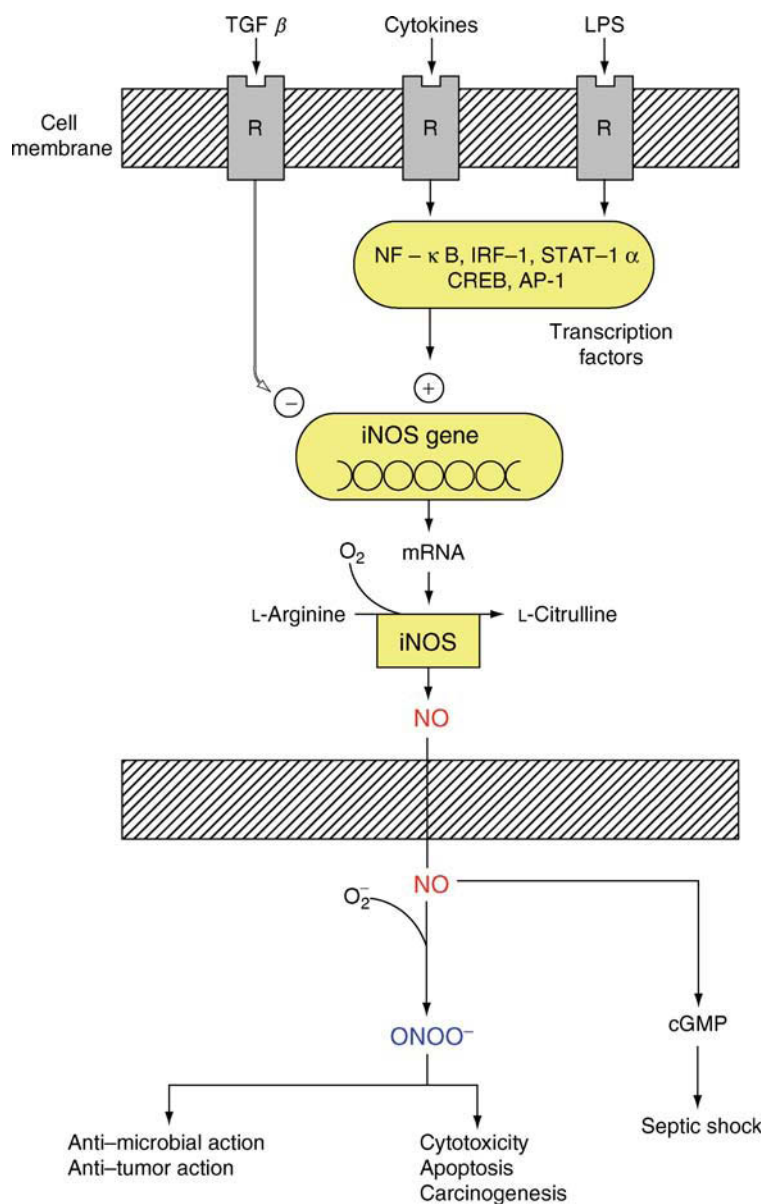
Unlike the NO pathways that regulate hippocampal LTP, cyclic GMP is not involved in NO toxicity. Excess stimulation of nNOS at the synapse has the potential to mediate neurotoxicity in the brain. Many causes of neural injury, including those associated with stroke and certain neurotoxins, are due to excess release of glutamate, which acts at synaptic NMDA receptors to cause neurotoxicity. As a free radical, NO is inherently reactive and mediates cellular toxicity by damaging critical metabolic enzymes and by reacting with superoxide anions to form an even more potent oxidant, peroxynitrite that participates in DNA damage, lipid peroxidation, protein nitration, and cellular energy depletion. Through these mechanisms, NO appears to play a major role in the pathophysiology of stroke, Parkinson's disease, Alzheimer's disease, and Huntington's disease.

NO Formed by iNOS

Under pathological conditions (e.g., during inflammation), high levels of NO are produced after induction of the expression of iNOS mainly in macrophages. NO possesses the protective/destructive duality inherent in every other major component of the immune response. On the one hand, it exerts beneficial effects by acting as an anti-bacterial, anti-parasitic, and anti-viral agent or as a tumoricidal agent; on the other hand, high levels of NO, if uncontrolled, elicits detrimental effects that are produced because NO reacts with concomitantly produced superoxide anions, thereby generating highly toxic compounds such as peroxynitrite (Fig. 3) and hydroxyl radicals. Suppression of mitochondrial respiration is one of the mechanisms underlying NO-induced cytotoxicity. Inducers of iNOS expression include bacterial polysaccharides and immune cytokines such as interferon- γ , tumor necrosis factor α , and interleukin (IL) 1 β . Suppressors of iNOS expression include dexamethasone, corticosteroids, nonsteroidal anti-inflammatory agents, estrogens, transforming growth factor β , IL-4, IL-8, IL-10, IL-11, and IL-13 [5]. Transcription factors and the sequential pathway involved in iNOS expression are shown in Fig. 3.

iNOS is not normally present in the brain and retina, but is detected mainly in glial cells of the brain and retina after inflammatory, infectious, or ischemic damage as well as in the aging brain. Excessive NO production by iNOS seems to contribute to the pathophysiology of many diseases that involve the central nervous system and eye.

Unphysiologically high levels of NO formed by iNOS expressed in tissues (e.g., liver, stomach, and lung) with chronic inflammation following infections of



Nitric Oxide. Figure 3 Possible pathways of iNOS induction associated with cytokines and lipopolysaccharides (LPS) in macrophages, glial cells, and smooth muscle cells and beneficial and detrimental actions of NO formed by iNOS. TGF- β_1 , transforming growth factor- β_1 ; R, receptor; +, stimulation; -, inhibition; O₂⁻, superoxide anion; ONOO⁻, peroxynitrite. Transcription factors: NF- κ B, nuclear factor- κ B; IRF-1, interferon regulatory factor-1; STAT-1 α , signal transducer and activator of transcription-1 α ; CREB, cyclic AMP-responsive element binding protein; AP-1, activating protein-1.

viruses (e.g., hepatitis and Epstein-Barr), parasites, or bacteria (*Helicobacter pylori*) may play a key role in carcinogenesis. Reactive nitrogen species, such as peroxynitrite, can damage DNA, RNA, lipids, and proteins, leading to increased mutations and altered functions of enzymes and proteins, and thus contributing to the multistage carcinogenesis process.

Excessive NO levels in plasma if being persistently associated with expression of iNOS in macrophages

and vascular smooth muscle cells result in fatal circulatory shock because of the remarkable fall of vascular resistance and venodilatation that involve profound hypotension and acute cardiac failure.

Pharmacological Relevance

Currently, one of the most well-known, promising therapeutic drugs in relation to endogenous NO is sildenafil. The main phosphodiesterase in the human

corpus cavernosum is type 5 cyclic GMP phosphodiesterase (►**phosphodiesterase-5 (PDE-5)**). Selective PDE-5 inhibitors, sildenafil, vardenafil, and tadalafil, enhance the erectile response to sexual stimulation in patients with erectile dysfunction [4]. This effect is associated with interference with the degradation by PDE-5 (Fig. 2) of cyclic GMP produced in cavernous smooth muscles through guanylyl cyclase that is activated by NO released from nitrergic nerves. Of course, care has to be taken to determine whether PDE-5 has been used before nitrates are administered to patients. There is some concern that patients may be adversely affected by an excessive drop of blood pressure in cases of their combined use.

Although the effect of L-arginine is too short to be clinically useful, the possibility for a novel therapeutic strategy with oral L-arginine has been raised. Dietary L-arginine improves endothelial function, inhibits platelet aggregation, reduces monocytes/endothelial cell adhesion in patients with coronary artery disease or heart failure, and lowers systemic blood pressure in patients with essential hypertension and women with preeclampsia. NO mainly synthesized by eNOS appears to participate in the beneficial effects of L-arginine that is evidenced from studies on experimental animals. L-Arginine contributes to improvement of the erectile function in some patients with erectile dysfunction. Treatment with L-arginine would be particularly efficient in patients with elevated plasma levels of the endogenous NOS inhibitor, asymmetric dimethyl-L-arginine.

►**Nitroglycerin** has long been used for the treatment of acute attack of angina pectoris, and its stable analogs are available to prevent the anginal attack. Nitrovasodilators such as sodium nitroprusside liberate NO from their molecules in the tissue (thus, called NO donors) and elicit actions via cyclic GMP like those seen with endogenously synthesized NO.

Inhaled NO has been used for treatment of persistent pulmonary hypertension of newborn infants, critical respiratory failure of preterm infants, and acute hypertension of adult cardiac surgery patients. PDE-5 inhibitors such as sildenafil are also effective for treatment of pulmonary hypertension. The combination of PDE-5 and NO inhalation yields additive beneficial effects on pulmonary hemodynamics. On the other hand, measurement of exhaled NO is a noninvasive and reproducible test that is a surrogate measure of airway inflammation in patients with bronchial asthma.

Findings obtained from experimental studies suggest that induction of iNOS mediates inflammatory or ischemic brain damage and that excessively activated nNOS under excitotoxic or ischemic conditions produces NO that is toxic to surrounding neurons. Selective inhibition of iNOS or nNOS may be neuroprotective. This is also the case in glaucoma and diabetic

retinopathy. Free radical scavengers may be effective in clinically preventing and treating neurological and psychological symptoms in patients after cerebral ischemic insult. In addition to scavenging free radicals generated by ischemia, these compounds preserve endothelial functions for the beneficial actions of NO in the brain and circulatory system.

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Nitric Oxide Synthase (NOS)

►NO Synthases

N-nitro-L-arginine Methyl Ester

►L-NAME

Nitrogen Mustards

The nitrogen mustards are cytotoxic chemotherapy agents which are chemically derived from mustard gas. Although their current use is medicinal, the predecessor of these compounds was also used for chemical warfare purposes.

►Alkylating Agents

Nitrosourea Derivatives

Nitrosourea derivatives are alkylating agents that include a nitroso (R–NO) group and a urea.

► Alkylating Agents

Nitrovasodilators

► Nitrates

NK Cells

► Natural Killer Cells

NK Receptors (Neurokinins Receptors)

► Tachykinins

NMDA

NMDA (*N*-methyl-D-aspartic acid) is a synthetic derivative of aspartic acid and represents the prototypical agonist at the NMDA receptors for which the latter were named.

- Psychotomimetic Drugs
- Ionotropic Glutamate Receptors

NMDA Receptor

NMDA receptors are subtypes of ionotropic glutamate receptors activated by NMDA forming heteromers

containing NRI (isoform 1–4) and NR2 (NR2A–NR2D) and in some cases NR3 subunits. They are permeable to Na⁺, K⁺ and Ca²⁺ ions. Inward ionic current through the receptor is voltage-dependent due to Mg²⁺ block at negative membrane potentials. Other families of ionotropic glutamate receptors are AMPA (α -amino-5-methyl-3-hydroxy-4-isoxazole propionic acid) and kainate receptors.

► Ionotropic Glutamate Receptors

NMR

NMR, nuclear magnetic resonance, is an analytical technique based on the energy differences of nuclear spin systems in a strong magnetic field. It is a powerful technique for structural elucidation of complex molecules.

- NMR-based Ligand Screening
- Combinatorial Chemistry

NMR-Screening

► SAR-by-NMR

NO

► Nitric Oxide

NO Donator

NO donators are a group of drugs, which are able to release NO. They include organic nitrates, sodium nitroprusside and molsidomine.

► Guanylyl Cyclases

NO Synthases

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Synonyms

Nitric oxide synthase

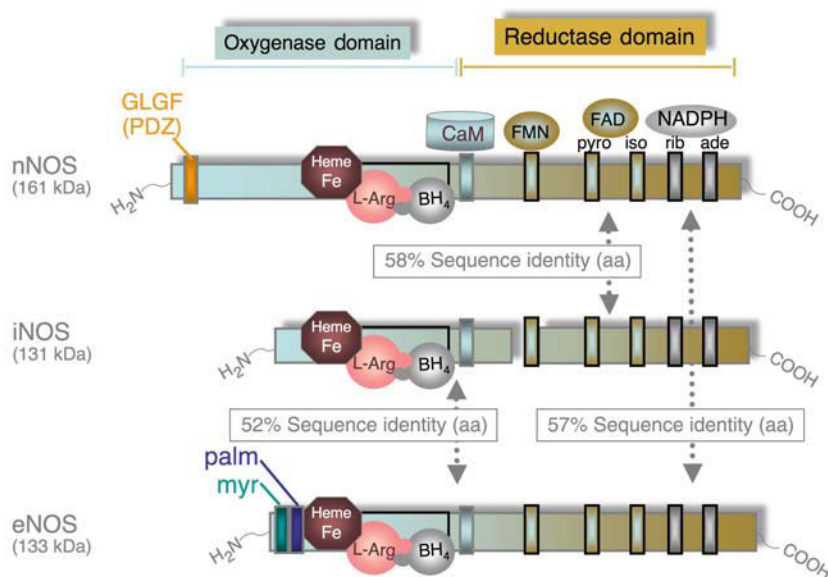
Definition

NO synthases (NOS, L-arginine, NADPH:oxygen oxidoreductases, nitric oxide forming; EC 1.14.13.39) represent a family of enzymes that catalyze the formation of nitric oxide (NO) from the amino acid L-arginine. In mammals, three isoforms of NOS have been identified. They are termed neuronal NOS (nNOS, NOS I, NOS1), inducible NOS (iNOS, NOS II, NOS2), and endothelial

NOS (eNOS, NOS III, NOS3). Classically, nNOS and eNOS were considered “constitutive” enzymes, whereas iNOS is cytokine-induced. Recent evidence suggests that nNOS and eNOS are also subject to important regulation of expression [1]. Within the human species, amino acid sequences of the three NOS isoforms share 52–58% identity. Each isoform is well conserved across mammalian species (>90% amino acid identity for nNOS and eNOS, >80% for iNOS). NOS enzymes exist in organisms as low as nematodes, protozoa, and even in plants (Fig. 1).

Basic Characteristics

All NOS isoforms utilize L-arginine as the substrate, and molecular oxygen and reduced nicotinamide adenine dinucleotide phosphate (NADPH) as cosubstrates. Flavin adenine dinucleotide (FMN), flavin mononucleotide (FAD), and (6R)-5,6,7,8-tetrahydro-L-biopterin (BH₄) are cofactors of the enzyme. All NOS isoforms contain heme and bind calmodulin. In nNOS and eNOS,



NO Synthases. Figure 1 Schematic diagram displaying the spatial relationships between the three cloned isoforms of NOS. Alignment of the deduced amino acid sequences of the three NOS isoforms revealed 50–60% sequence identity between the enzymes. All NOS isoforms consist of a reductase domain and an oxygenase domain. The reductase domain of the enzymes shows about 35% sequence identity with cytochrome P₄₅₀ reductase, and this enzyme shares the cofactor binding regions of the NOS for reduced nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Consensus sequences for the binding of the cosubstrate NADPH (adenine and ribose), FAD (isoalloxazine and pyrophosphate), FMN, calmodulin (CaM) and heme are indicated. The proximal part of the N-terminal oxygenase domain (black box) shows 65–71% sequence identity between the three NOS isoforms and contains the closely linked binding sites for L-arginine (L-Arg) and (6R)-5,6,7,8-tetrahydro-L-biopterin (BH₄). The C-terminal portion of the isozymes (reductase domain) is responsible for the NADPH diaphorase activity that is common to all three isozymes. nNOS contains an N-terminal tail that includes a GLGF (glycine, leucine, glycine, phenylalanine)-motif or PDZ (postsynaptic density protein 95/discs large/ZO-1 homology)-domain. This motif targets nNOS to other cytoskeletal proteins in brain and skeletal muscle. eNOS contains N-terminal myristoylation (myr) and palmitoylation (palm) sites. These lipid anchors contribute to the membrane localization of this isozyme.

calmodulin binding is brought about by an increase in intracellular Ca^{2+} (half-maximal activity between 200 and 400 nM). This leads to an enhanced binding of CaM to the enzyme, which in turn displaces an autoinhibitory loop and facilitates the flow of electrons from NADPH in the reductase domain to the heme in the oxygenase domain. In iNOS, calmodulin already binds at low intracellular Ca^{2+} concentrations (below 40 nM) due to a different amino acid structure of the calmodulin-binding site. In NOS, a zinc ion is coordinated tetrahedrally to pairs of cysteine motifs (CXXXXC), one motif being contributed by each NOS monomer. The C-terminal portion of all three NOS isozymes conveys ►**NADPH-diaphorase** activity. The NO formed by NOS can act on a number of target enzymes and proteins. The most important signaling pathway stimulated by NO is the activation of soluble guanylyl cyclase and the generation of cyclic GMP (Fig. 2).

Neuronal NO Synthase

Neuronal NO synthase (nNOS) is constitutively expressed in neurons of the brain. Its activity is regulated by Ca^{2+} and calmodulin. Half-saturating L-arginine concentrations are around 2 μM . cDNAs encoding nNOS have been cloned from rat and human brain. The open reading frame of human nNOS consists of 4299 bp, corresponding to 1433 aa. This predicts a protein of 160 kDa, which is in accordance with the molecular mass of the purified protein.

nNOS is not only found in brain. Immunochemical studies identified the enzyme in the spinal cord, in sympathetic ganglia and adrenal glands, in peripheral nitrergic nerves, in epithelial cells of lung, uterus, and stomach, in kidney macula densa cells, in pancreatic islet cells, and in vascular smooth muscle. In terms of tissue mass, the largest source of nNOS in mammals is skeletal muscle.

The gene for human nNOS is located in the 12q24.2–24.31 region of chromosome 12. The nNOS gene is by far the largest of the three NOS genes, spanning over 150 kb of genomic DNA. The mRNA is encoded by 29 exons with translation start and stop sites in exons 2 and 29, respectively. More than ten different exons 1 have been identified, which are mostly spliced to a common exon 2. Moreover, skeletal muscle and some other tissues express an elongated protein (nNOS μ), containing a 102-bp cassette exon insertion between exons 16 and 17 of the human nNOS gene. There is no evidence for a difference in function between “brain-type” nNOS and “muscle type” nNOS μ .

Functions of nNOS include long-term regulation of synaptic transmission in the CNS (long-term potentiation, long-term depression), whereas there is no evidence for an involvement of nNOS-derived NO in acute neurotransmission. Retrograde communication across synaptic junctions is presumed to be involved in memory

formation, and there is evidence that inhibitors of NOS impair learning and can produce amnesia in animal models. Evidence is also accumulating that NO formed in the CNS by nNOS is involved in the central regulation of blood pressure. In the periphery, many smooth muscle tissues are innervated by nitrergic nerves, i.e. nerves that contain nNOS and generate and release NO. NO produced by nNOS in nitrergic nerves can be viewed as an unorthodox neurotransmitter that decreases the tone of various types of smooth muscle. By mediating the relaxation of corpus cavernosum smooth muscle, nNOS is responsible for penile erection. In addition, in the corpus cavernosum, NO-induced smooth muscle relaxation is mediated by cyclic GMP. Cyclic GMP is degraded by ►**phosphodiesterases**, in corpus cavernosum particularly the isoform 5. Thus, nNOS activity represents the basis of the effect of the clinically used ►**phosphodiesterase 5** inhibitors sildenafil (Viagra®), vardenafil (Levitra®), and tadalafil (Cialis®).

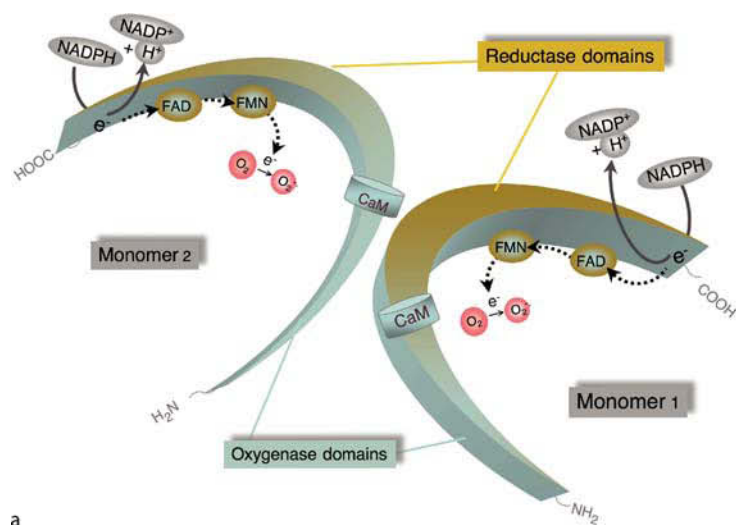
On the pathophysiological side, hyperactive nNOS has been implicated in *N*-methyl-D-aspartate (NMDA)-receptor-mediated neuronal death in cerebrovascular stroke. Some disturbances of smooth muscle tone within the gastrointestinal tract (e.g., gastroesophageal reflux disease) may also be related to an overproduction of NO by nNOS in peripheral nitrergic nerves.

Inducible NO Synthase

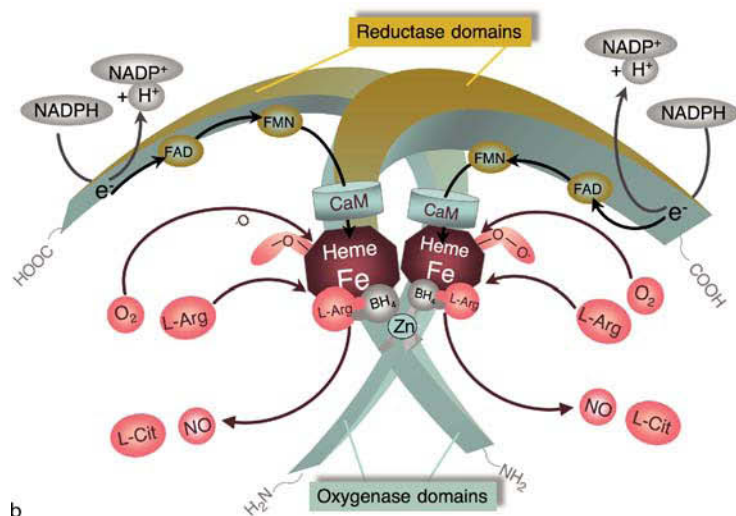
Inducible NO synthase (iNOS) is usually not constitutively expressed, but can be induced in macrophages by bacterial lipopolysaccharide (LPS), cytokines and other agents. Although primarily identified in macrophages, expression of the enzyme can be stimulated in virtually any cell or tissue, provided the appropriate inducing agents have been identified (for review see [1] and [3]).

Once expressed, iNOS is active and not regulated by intracellular Ca^{2+} concentration. Half-saturating L-arginine concentrations for iNOS have been reported between 3 and 30 μM . cDNAs encoding iNOS have been cloned from murine, rat, and human cells and tissues. The open reading frame of human iNOS is 3459 bp, corresponding to 1153 aa and predicting a protein of 131 kDa, which is in agreement with data obtained for purified iNOS. The human gene for iNOS has been localized to the 17p11–17q11 region of chromosome 17. The gene contains 26 exons and spans 37 kb of DNA.

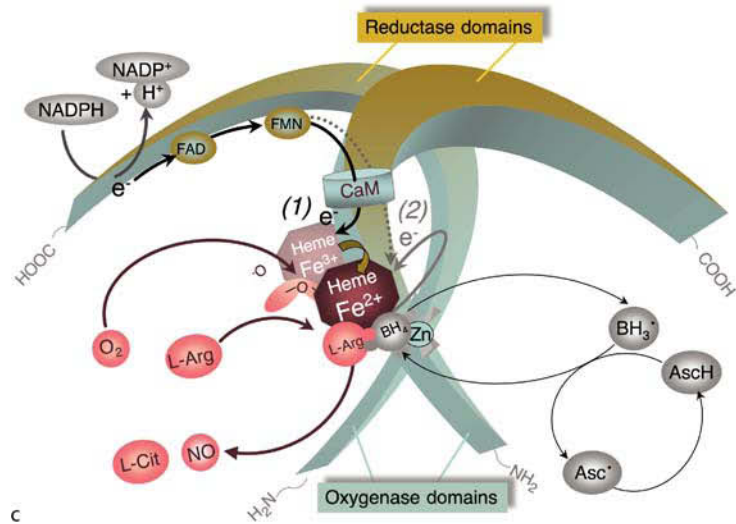
When induced in macrophages, iNOS produces large amounts of NO which represents a major cytotoxic principle of those cells. Due to its affinity to protein-bound iron, NO can inhibit a number of key enzymes that contain iron in their catalytic centers. These include ribonucleotide reductase (rate-limiting in DNA replication), iron–sulfur cluster-dependent enzymes (complex I and II) involved in mitochondrial electron transport and cis-aconitase in the citric acid cycle. In addition, higher concentrations of NO,



a

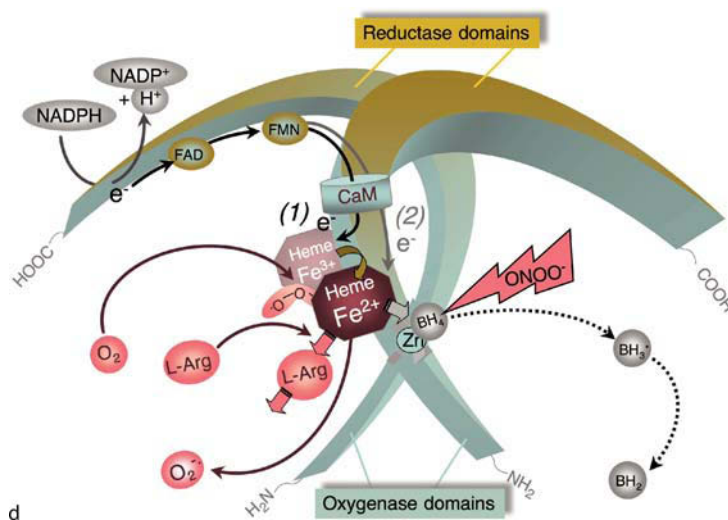


b



C

NO Synthases. Figure 2 (Continued)



NO Synthases. Figure 2 Schematic diagrams showing NOS catalysis under normal conditions and in pathophysiology. (a) NOS monomers and even isolated reductase domains are capable of transferring electrons from reduced nicotinamide-adenine-dinucleotide phosphate (NADPH), to flavin-adenine-dinucleotide (FAD) and flavin mononucleotide (FMN) and have a limited capacity to reduce molecular oxygen to superoxide ($O_2^{\bullet-}$). Monomers and isolated reductase domains can bind calmodulin (CaM), which stimulates electron transfer within the reductase domain. NOS monomers are unable to bind the cofactor (6*R*)-5,6,7,8-tetrahydrobiopterin (BH_4) or the substrate L-arginine and cannot catalyze NO production. (b) The presence of heme allows for NOS dimerization; heme is the only cofactor required for the formation of active NOS dimers. Heme is also essential for the interaction between reductase and oxygenase domains and for the calmodulin-dependent interdomain electron transfer from the flavins to the heme of the opposite monomer. Due to differences in the CaM-binding domain, elevated Ca^{2+} is required for CaM binding (and thus catalytic activity) in nNOS and eNOS, whereas CaM binds to iNOS with high affinity even in the absence of Ca^{2+} . When sufficient substrate L-arginine (L-Arg) and cofactor BH_4 are present, intact NOS dimers couple their heme- and O_2 reduction to the synthesis of NO (fully functional NOS). L-citrulline is formed as the byproduct. NOS enzymes perform two separate oxidation steps, one to form ω -hydroxy-L-arginine and a second to convert this intermediate to NO. All NOS isoforms contain a zinc ion (Zn) coordinated in a tetrahedral conformation with pairs of CXXXXC-motifs at the dimer interface. This site is also of major importance for the binding of BH_4 and L-arginine. (c) Electron transfer from the reductase domain (1) enables NOS ferric (Fe^{3+}) heme to bind O_2 and form a ferrous (Fe^{2+})-dioxy species. This species may receive a second electron preferentially from BH_4 or from the reductase domain (2). The nature of the resulting oxidized BH_4 has been identified by electron paramagnetic resonance as the trihydrobiopterin radical ($BH_3^{\bullet}$) or the trihydropterin radical cation protonated at N5 ($BH_3^{\bullet}H^+$). The $BH_3^{\bullet}$ radical (or radical cation) can be recycled to BH_4 by the NOS itself (using an electron supplied by the flavins). Alternatively, there is evidence that reducing agents such as ascorbic acid (AscH, which is present in cells in millimolar concentrations) can reduce the $BH_3^{\bullet}$ radical back to BH_4 (Asc = ascorbate radical). (d) Under pathophysiological conditions, all NOS enzymes can uncouple their oxygen reduction from NO synthesis. An established condition under which this occurs is oxidative stress. In this situation superoxide ($O_2^{\bullet-}$) generated by other oxidases can combine with NO generated by a still functional NOS. This leads to an increased formation of peroxynitrite ($ONOO^-$), which – in turn – can oxidize BH_4 to biologically inactive products such as the $BH_3^{\bullet}$ radical or 6,7-[8H]-H₂-biopterin (BH_2). In the absence of functional BH_4 , also the affinity of the substrate L-arginine (L-Arg) to the NOS is reduced, and NOS catalyzes the uncoupled reduction of O_2 , leading to the production of $O_2^{\bullet-}$ (and possibly also H_2O_2).

as produced by induced macrophages, can directly interfere with the DNA of target cells and cause strand breaks and fragmentation. A combination of these effects is likely to form the basis of the cytostatic and cytotoxic effects of NO on parasitic microorganisms and tumor cells.

However, the high levels of NO produced by activated macrophages (and probably neutrophils and

other cells) may not only be toxic to undesired microbes, parasites, or tumor cells, but, when released at the wrong site, may also harm healthy cells. In vivo, cell and tissue damage can be related to the NO radical (NO) itself or an interaction of NO with superoxide leading to the formation of peroxynitrite ($ONOO^-$). The large majority of inflammatory- and autoimmune lesions are characterized by an abundance of activated

macrophages and neutrophils. High levels of NO can be secreted by those cells, leading to damage of the surrounding tissue. Interestingly, nonimmune cells can also be induced with cytokines to release amounts of NO large enough to affect neighboring cells. Cytokine-activated endothelial cells have been shown to lyse tumor cells, pancreatic islet endothelial cells can be induced to destroy adjacent β -cells, and induced hepatocytes can use NO to kill malaria sporozoites. iNOS activity is likely to be responsible for all of these effects. Additionally, tissue damage produced in animal models of immune complex alveolitis and dermal vasculitis is likely to depend on the presence of excess NO. iNOS-derived NO seems to be involved also in nonspecific allograft rejection. Finally, iNOS is likely to play an important role in septic shock. This disease is characterized by massive arteriolar vasodilatation, hypotension and microvascular damage. Bacterial endotoxins initiate the symptoms. A number of mediators such as platelet-activating factor, thromboxane A_2 , prostanooids, and cytokines such as interleukin-1, tumor necrosis factor- α , and interferon- γ are elevated in septic shock and have been implicated in its pathophysiology. However, the fall in blood pressure is largely due to excess NO production by iNOS induced in the vascular wall, as demonstrated in experiments with NOS inhibitors. Similarly, interleukin-2 therapy is complicated by hypotension, and iNOS induction in response to cytokines may also take place in the vascular system of these patients.

Endothelial NO Synthase

Endothelial NO Synthase (eNOS) expression is relatively specific for endothelial cells. However, the isozyme has also been detected in certain neurons of the brain, in syncytiotrophoblasts of human placenta and in LLC-PK₁ kidney tubular epithelial cells.

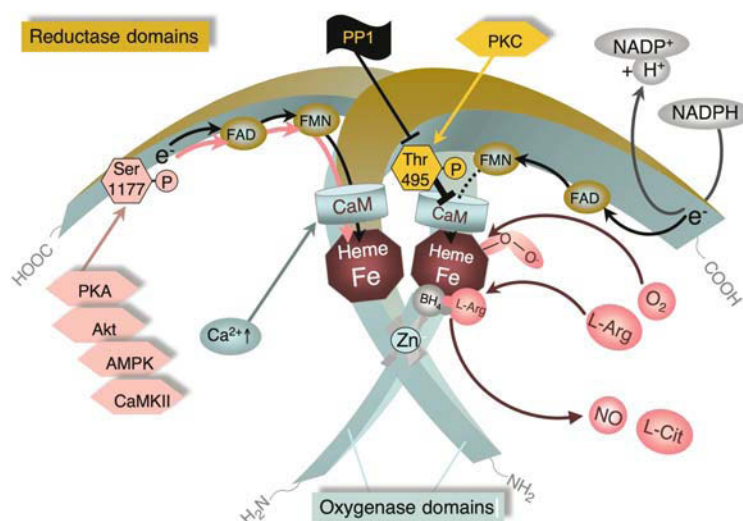
The cDNAs encoding eNOS have been cloned from bovine and human endothelial cells. The open reading frame of human eNOS encompasses 3609 bp, corresponding to 1203 aa and predicting a protein of 133 kDa, which is in good agreement with the molecular mass determined for the purified protein. Its activity is regulated by Ca^{2+} and calmodulin. Half-saturating concentrations for L-arginine are around 3 μ M. The human endothelial NOS gene has been localized to the 7q35–7q36 region of chromosome 7. The gene contains 26 exons spanning about 22 kb of genomic DNA.

Similar to nNOS, Ca^{2+} -activated calmodulin is important for the regulation of eNOS activity. However, several other proteins interact with eNOS and regulate its activity. Heat shock protein 90 (hsp90) is found associated with eNOS and probably acts as an allosteric modulator that activates the enzyme. Caveolin-1 binds eNOS and directs it to caveolae. Caveolin-1 is viewed as an inhibitor of eNOS activity, which is being replaced by CaM upon activation of endothelial cells [2].

In addition, eNOS is subject to protein phosphorylation. It can be phosphorylated on several serine (Ser), threonine (Thr), and tyrosine (Tyr) residues; however, major changes in enzyme function have been reported for the phosphorylation of amino acid residues Ser1177 and Thr495 (in the human eNOS sequence) (Fig. 3).

Phosphorylation of Ser1177 is induced when endothelial cells are exposed to fluid shear stress, estrogens, vascular endothelial growth factor (VEGF), insulin, or bradykinin. Shear stress elicits phosphorylation by activating protein kinase A, estrogen, and VEGF phosphorylate eNOS mainly via the serine/threonine kinase Akt, insulin probably activates both Akt and the AMP-activated protein kinase, and the bradykinin-induced phosphorylation of Ser1177 is mediated by Ca^{2+} /CaM-dependent protein kinase II. Phosphorylation of Ser1177 stimulates the flux of electrons within the reductase domain, increases the Ca^{2+} sensitivity of the enzyme, and represents an additional and independent mechanism of eNOS activation (for review see [2] and [4]). Phosphorylation of Thr495, on the other hand, decreases enzymatic activity. This site tends to be phosphorylated under nonstimulated conditions (most probably by protein kinase C). Phosphorylation of Thr495 is likely to interfere with the binding of CaM to the CaM-binding domain. The phosphatase that dephosphorylates Thr495 appears to be protein phosphatase 1. Dephosphorylation of Thr495 has been seen when endothelial cells were stimulated with agents such as histamine or bradykinin (that also elevate intracellular Ca^{2+} concentrations). Substantially more CaM binds to eNOS when Thr495 is dephosphorylated (for review see [2] and [4]). However, dephosphorylation of Thr495 has also been shown to favor uncoupling of oxygen reduction from NO synthesis in eNOS (see above). Other phosphorylation sites of human eNOS exist, but are not known to have major consequences for enzyme activity.

In addition to its vasodilator properties, eNOS-derived NO can convey vasoprotection in several ways. NO released toward the vascular lumen is a potent inhibitor of platelet aggregation and adhesion to the vascular wall. Besides protection from thrombosis, this also prevents the release of platelet-derived growth factors that stimulate smooth muscle proliferation and its production of matrix molecules. Endothelial NO also controls the expression of genes involved in atherogenesis. NO decreases the expression of chemoattractant protein MCP-1 and of a number of surface adhesion molecules thereby preventing leukocyte adhesion to vascular endothelium and leukocyte migration into the vascular wall. This offers protection against an early phase of atherogenesis. Additionally, the decreased endothelial permeability, the reduced influx of lipoproteins into the vascular wall, and the inhibition of low density lipoprotein (LDL) oxidation may contribute to the



NO Synthases. Figure 3 Schematic diagram showing phosphorylation of eNOS by various kinases. Functionally important phosphorylation sites in human eNOS are Ser1177 (pink hexagon) and Thr495 (yellow hexagon). Phosphorylation of Ser1177 enhances electron flux in the reductase domain (pink arrows) and increases eNOS activity. It is brought about by protein kinase A (PKA), protein kinase Akt, AMP-activated protein kinase (AMPK), and Ca^{2+} /CaM-dependent protein kinase II (CaMKII) (pink hexagons). Thr495 tends to be phosphorylated under nonstimulated conditions (most probably by protein kinase C, PKC, yellow hexagon). Phosphorylation of Thr495 reduces eNOS activity (black block arrow). The phosphatase that dephosphorylates Thr495 appears to be protein phosphatase 1 (PP1, black flag with black block arrow).

antiatherogenic properties of eNOS-derived NO. Furthermore, NO has been shown to inhibit DNA synthesis, mitogenesis, and proliferation of vascular smooth muscle cells as well as smooth muscle cell migration, thereby protecting against a later phase of atherosclerosis. Based on the combination of those effects, NO produced in endothelial cells can be considered an antiatherosclerotic principle (for review see [4] and [5]).

Impaired NO-mediated vasodilatation has been seen in hypercholesterolemia and atherosclerosis. Arteries, especially human coronary arteries, may thereby be predisposed to vasoconstriction and vasospasm. Indeed, the paradoxical vasoconstriction in response to the endothelium-dependent vasodilator acetylcholine can be used as a diagnostic indicator of beginning coronary atherosclerosis during coronary catheterization. Reduced eNOS-mediated vasodilatation has also been found in arteries from hypertensive and diabetic animals, and impaired responses to endothelium-dependent vasodilators has been demonstrated in the forearm of hypertensive patients. In isolated blood vessels from animals with pathophysiological conditions such as hypertension, diabetes, or nitroglycerin tolerance, evidence has been obtained for eNOS uncoupling. Under these conditions, superoxide is generated from the oxygenase domain instead of NO. Simultaneous addition of L-arginine and BH_4 restores NO production and abolishes superoxide generation by eNOS in vitro. Administration of BH_4 also restored endothelial function in animal models of

experimental diabetes and insulin resistance, as well as in patients with hypercholesterolemia and in smokers. Oxidative stress occurs in pathological conditions such as hypercholesterolemia, diabetes, aging and smoking, and oxidation of BH_4 may be the common cause of eNOS dysfunction in these situations (for review see [4] and [5]).

- Guanylyl Cyclase
- Smooth Muscle Tone Regulation

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Nociceptin/Orphanin FQ

Nociceptin is an opioid peptide, which is the endogenous ligand for the opioid receptor-like protein (ORL).

► Opioid System

generally described as ‘pricking’, whereas that evoked by activation of C-fibres is generally described as ‘burning’.

- Local Anaesthetics
- Analgesics
- Pain and Nociception

Nociception

Nociception is the normal experience of pain in healthy people.

- Galanin Receptors
- Pain and Nociception

Nociceptive Transducers

Nociceptive transducers are ion-channel complexes, which generate depolarising currents in nociceptors in response to specific noxious stimuli. Transducer proteins for irritant and chemical stimuli include the vanilloid receptors VR1 and VRL1 (cation-channels gated by protons, capsaicin and noxious heat, i.e. 46°C), the purinergic receptor P2X3 (ATP-gated ion channel) and acid-sensing ion channels such as ASIC- α , ASIC- β and DRASIC (proton-gated ion-channels). The molecular identity of the transducers for noxious mechanical stimuli is as yet unknown. Expression of nociceptive transducers is altered following tissue injury or disease, which contributes to long-term sensitization of nociceptors.

► Nociception

Nociceptors

Nociceptors are a specific subset of peripheral sensory organs, which respond to noxious stimuli. A δ mechanoreceptors and C-polymodal nociceptors are the two main classes of cutaneous nociceptors. The sensory quality of pain evoked by activation of A δ -fibres is

Nodes of Ranvier

The nodes of Ranvier are the gaps formed between myelin sheath cells along the axons. The sodium channels are densely localized at the nodes of Ranvier.

Non-adrenergic Non-cholinergic (NANC) Transmission/Mediators

Non-adrenergic non-cholinergic (NANC) transmission/mediators describes a part of the autonomic nervous system which does not use acetylcholine or noradrenaline as transmitters. NANC-transmitters often function as co-transmitters, which are released together with acetylcholine or noradrenaline. Substances believed to function as NANC transmitters include ATP, which is found e.g. in postganglionic sympathetic neurons of blood vessels and may contribute to the fast contraction of smooth muscle cells, γ -aminobutyric acid (GABA) and serotonin which are found in enteric neurons and are involved in peristaltic reflexes, dopamine, found in some sympathetic neurons (e.g. kidney) and involved in vasodilatation and NO which is released from pelvic or gastric nerves and plays a role in penile erection or gastric emptying. A variety of peptides have also been described to function as NANC transmitters, e.g. neuropeptide Y (postganglionic sympathetic neurons), vasoactive intestinal peptide (parasympathetic nerves to salivary glands), substance P (sympathetic ganglia, enteric neurons) or calcitonin gene-related peptide (CGRP) (non-myelinated sensory neurons).

► Synaptic Transmission

Non-competitive Antagonism

If an antagonist binds to the receptor and precludes agonist activation of that receptor by its occupancy, then

no amount of agonist present in the receptor compartment can overcome this antagonism and it is termed noncompetitive. This can occur either by binding to the same binding domain of the agonist or another (allosteric) domain. Therefore, this definition is operational in that it does not necessarily imply a molecular mechanism, only a cause and effect relationship. The characteristic of noncompetitive antagonism is eventual depression of the maximal response, however, parallel displacement of agonist dose–response curves, with no diminution of maximal response, can occur in systems with receptor reserve for the agonist.

► Drug–Receptor Interaction

Non-competitive Inhibition

Reversible metabolic inhibition caused by an inhibitor binding to an enzyme site different from the substrate. The degree of inhibition is independent of the substrate concentration.

► Drug Interaction

Nonionic Contrast Media

Nonionic contrast media are neutral water-soluble triiodobenzene derivatives; the solubility is provided by hydrophilic groups in the side chains, preferentially hydroxy groups.

► Radiocontrast Agents

Non-neuronal Monoamine Transporters

Non-neuronal monoamine transporters is the collective designation for OCT1, OCT2, and EMT; the term indicates that these three carriers share some substrates with the neuronal monoamine transporters (such as DAT, NET, SERT) and the vesicular monoamine

transporters (VMAT), but they are expressed (at least predominantly) in non-neuronal cells (e.g. in glia cells, hepatocytes or in the proximal tubule).

- Organic Cation Transporters
- Vesicular Transporters

Non-opioid Analgesics

Non-opioid analgesics can be divided into two groups. The first group contains substances having anti-inflammatory effects in addition to their analgesic and antipyretic activity and is called non-steroidal anti-inflammatory drugs (NSAIDs). The second group of non-opioid analgesics, which are not classified as NSAIDs, consists of substances that lack anti-inflammatory properties, such as phenazones, metamizole (dipyrone) and paracetamol.

- COX-2
- Cyclooxygenases
- Analgesics
- Non-steroidal Anti-inflammatory Drugs
- Prostaglandins
- Prostanoids

Non-rapid Eye Movement (NREM) Sleep

As the name suggests, NREM sleep describes collectively all sleep activity that does not class as REM sleep. In humans, it is subdivided into *sleep stages* 1–4; stage 1, representing the lightest stage of sleep (often experienced as drowsiness), and stage 4 the deepest sleep, from which it is most difficult to wake the sleeper. On the EEG, NREM sleep is characterised by the presence of slow waves (i.e. low frequency, high amplitude activity), the relative proportion of which increases as sleep deepens. In contrast to REM sleep, no specific movement of the eyeballs is detectable, and no muscle atonia is present. It is though that dreaming occurs primarily during REM sleep, based on reports of subjects woken at various points during the sleep–wake cycle, although some dreaming activity was found to occur during NREM sleep.

► Sleep

Nonreceptor Tyrosine Kinases

► Tyrosine Kinases

Non-selective Cation Channels

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Definition

Non-selective cation channels are macromolecular pores in the cell membrane that form an aqueous pathway. These enable cations such as Na^+ , K^+ or Ca^{++} to flow rapidly, as determined by their ►electrochemical driving force, at roughly equal rates ($>10^7$ cations per channel pore and per second).

Basic Characteristics

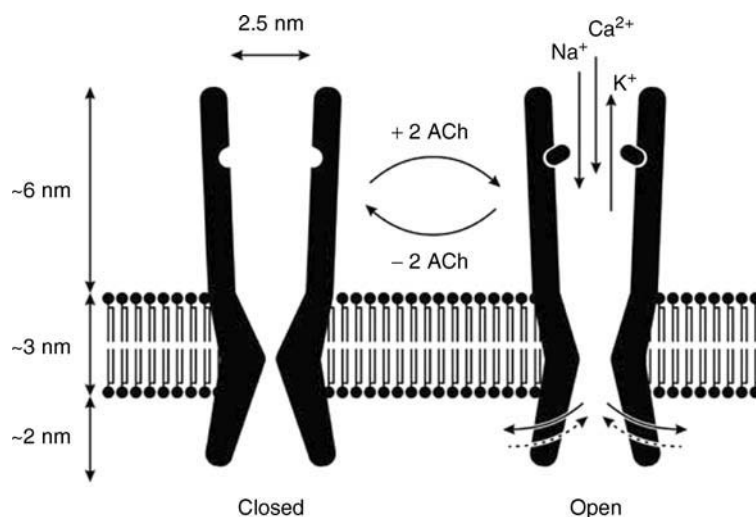
In general, ion channels are multi protein complexes residing in cellular membranes and allowing ions, mainly Na^+ , K^+ , Ca^{++} and Cl^- , to flow rapidly as determined by their electrochemical driving force in a thermodynamically downhill direction. The channel proteins are dynamic structures; they form channels that can exist in at least two conformational states, open and closed. In the closed state the channel can be inactive and/or is reluctant to be activated. Fast shifts in the submillisecond range between these states can be regulated by the membrane potential (►voltage gated ion channels), by ligands (►ligand gated ion channels) or agonist. In many cases these shifts can be modulated on a longer time scale (milliseconds to minutes) by hormones, neurotransmitters, drugs and toxins.

The open channel has in most cases a selective permeability, allowing a restricted class of ions to flow, for example Na^+ , K^+ , Ca^{++} or Cl^- and, accordingly, these channels are called Na^+ -channels, K^+ -channels, Ca^{++} -channels and Cl^- -channels. In contrast, cation-permeable channels with little selectivity reject all anions but discriminate little among small cations. Little is known about the structures and functions of these non-selective cation channels [1], and so far only one of them, the ►nicotinic acetylcholine receptor (nAChR, see Nicotinic Receptors), has been characterized in depth [2, 3]. The nAChR is a ligand-gated channel (see below) that does not select well among cations; the channel is even permeable to choline, glycine ethylester and tris buffer cations. A number of other plasma

membrane cation channels including certain ►ionotropic glutamate receptors, the ►capsaicin receptor and ►cyclic nucleotide-gated channels are also sometimes called non-selective; they favour the flow of Ca^{++} over Na^+ with relative permeabilities of <4 (►glutamate receptors of the NMDA-type), <10 (capsaicin receptor) and <90 (CNG channels). Others, like ►hyperpolarization-activated and cyclic nucleotide-gated channels conduct both K^+ and Na^+ , but are impermeable to divalent cations. Yet another group of channels, which belong to the superfamily of ►TRP channels [4], have been implicated to represent non-selective cation channels. However, their mode of gating and, most importantly, their pore regions have not been determined and characterized as yet. There are two exceptions: The capsaicin receptor, which is mentioned above as slightly Ca^{++} selective channel and the ►epithelial Ca^{++} channel 1 or ECAC1 (synonym TRPV5). The latter channel is highly selective for Ca^{++} compared to Na^+ with a relative permeability above 100. Finally, a subset of channels activated by stretch have been implicated to be non-selective cation channels [1]. These channels appear to be ubiquitous, but their structures are unknown.

Acetylcholine-activated channels are found in the membrane of vertebrate skeletal muscle at the synapse between nerve and muscle, also called the neuromuscular junction (muscle type nAChR). Their function is to depolarize the postsynaptic muscle membrane when the presynaptic nerve terminal releases its chemical neurotransmitter, acetylcholine. The channel is composed of four protein subunits that are assembled in a predetermined arrangement and stoichiometry ($\alpha 2\beta\gamma\delta$) around a central, cation-selective pathway. Transmembrane segments of the AChR subunits like those of other channels are mainly formed by 19–21 amino acid residues that are folded into a α helix. The α helix is a rod-like structure, the tightly coiled polypeptide main chain of which forms the inner part of the rod, and the side chains extend outward in a helical array [5]. Each residue is related to the next one by a rise of 0.15 nm along the helix axis and a rotation of 100° , which gives 3.6 amino acid residues per turn of helix. Accordingly, one turn of the helix is 0.54 nm, which is equal to 0.15 times the number of residues per turn. The hydrocarbon core of cellular membranes is typically 3 nm wide, which accordingly, can be traversed by a α helix consisting of 19–21 residues.

The nAChR is cylindrical with a mean diameter of about 6.5 nm (Fig. 1). All five rod-shaped subunits span the membrane. The receptor protrudes by <6 nm on the synaptic side of the membrane and by <2 nm on the cytosolic side [2]. The pore of the channel is along its symmetry axis and includes an extracellular entrance domain, a transmembrane domain and a cytosolic entrance domain. The diameter of the extracellular entrance domain is <2.5 nm and it becomes narrower at the transmembrane domain. The



Non-selective Cation Channels. Figure 1 The nicotinic acetylcholine receptor (nAChR) is localized within the cell membrane; above the cell membrane is the synaptic cleft, below the cytoplasm. Drawing of the closed (left) and open (right) nAChR showing acetylcholine (ACh) binding and cation movement. Dimensions of the receptor were taken from references [2, 3].

pore is lined by five α -helices, one from each subunit, and adjacent extended loop regions [3]. If two ACh molecules bind to the receptor sites at the extracellular surface of the receptor, far from each other and from the pore, this pathway opens, allowing permeation of Na^+ (crystal radius 0.095 nm) K^+ (crystal radius 0.133 nm) and Ca^{++} (crystal radius 0.099 nm), and initiates depolarization.

Monovalent or divalent cations, but not anions, readily flow through the open form of the AChR channel. What makes the channel cation selective? The amino acid sequences of the pore-forming helices and the adjacent loop components contain three rings of negatively charged residues. One of them is located within the transmembrane region of the pore, and the other two flank the cytosolic entrance to the pore. Apparently, the upper part of the channel, namely the α -helical components act as a water pore, whereas the lower loop components contribute to the selectivity filter of the channel [3]. Anions, such as Cl^- cannot enter the pore because they are repelled by the negatively charged rings. Studies on the permeability of a series of organic cations differing in size, such as alkylammonium ions, triaminoguanidinium, histidine and choline, indicate that the narrowest part of the pore has the dimension of <0.65 nm by 0.65 nm.

At the level of a [single channel](#), addition of ACh is followed by transient openings of the channel. The current i flowing through an open channel is 4 pA at a membrane potential V of -100 mV. Since one ampere (A) represents the flow of 6.24×10^{18} charges per second, 2.5×10^7 Na^+ ions per second flow through an open channel. The conductance g of a plasma membrane channel is the measure of the ease of flow of current between the extracellular space and the cytosol or vice

versa and is equal to $i/(V-E_r)$, where E_r is the reversal potential at which there is no ionic net flux; g is expressed in units of siemens (the reciprocal of an ohm), i in amperes, and V in volts. E_r equals 0 mV for non-selective cation channels, thus, a current of 4 pA at a potential of 100 mV corresponds to a conductance of 40 ps.

►TRP Channels

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Non-selective Monoamine Reuptake Inhibitor

Non-selective monoamine reuptake inhibitors (NSMRI) are a group of antidepressants, which function by inhibiting the reuptake of noradrenaline

and serotonin from the synaptic cleft by blockade of the neurotransmitter transporters specific for noradrenaline and serotonin. Most tricyclic antidepressants are non-selective monoamine reuptake inhibitors (e.g. imipramine, amitriptyline, desipramine).

- Antidepressants
- Synaptic Transmission

Non-Specific ChE

- Cholinesterases

Non-steroidal Anti-inflammatory Drugs

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Synonyms

NSAIDs; Non-steroidal anti-rheumatic drugs; NSAR
(aspirin-like drugs, Inflammation)

Definition

► **Non-opioid analgesic** agents can be divided into two groups. The first group contains substances having anti-inflammatory effects in addition to their analgesic and anti-pyretic activity and are called non-steroidal anti-inflammatory drugs (NSAIDs). The members of this group, with the exception of almost all selective inhibitors of cyclooxygenase 2 (► **COX-2**), are acids. Acidic NSAIDs, which include salicylates, derivatives of acetic acid and propionic acid and oxicams among others, comprise molecules containing a lipophilic and a hydrophilic region and are more than 99% bound to plasma proteins.

The second group of non-opioid analgesics, which are not classified as NSAIDs, consists of substances that lack anti-inflammatory properties, such as phenazones, metamizole (dipyrone) and paracetamol. Their molecules are neutral or weakly basic, have no hydrophilic

polarity, and are much less strongly bound to plasma proteins than NSAIDs.

Mechanism of Action

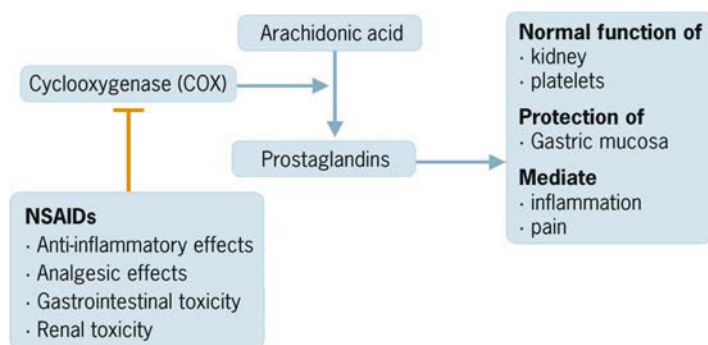
In the 1970s, NSAIDs were shown to interfere with the biosynthesis of ► **prostaglandins** [1]. NSAIDs block cyclooxygenases (COX) that catalyze the formation of cyclic endoperoxides from arachidonic acid (Fig. 1). Cyclic endoperoxides are precursors of the prostaglandins, thromboxane-A₂ and prostacyclin. Prostaglandins have a major role in the pathogenesis of pain, fever and inflammation. Inhibition of their biosynthesis would therefore be expected to result in analgesic, anti-pyretic and anti-inflammatory activity. However, since prostaglandins are synthesised in most tissues and have a variety of physiological functions, inhibition of their biosynthesis also causes unwanted effects. The clinically most important of these are gastrointestinal erosion and ulceration with bleeding and perforation and kidney disorders with retention of sodium ions and water.

Moreover, there are COX-independent effects of NSAIDs potentially contributing to the activity of NSAIDs [2].

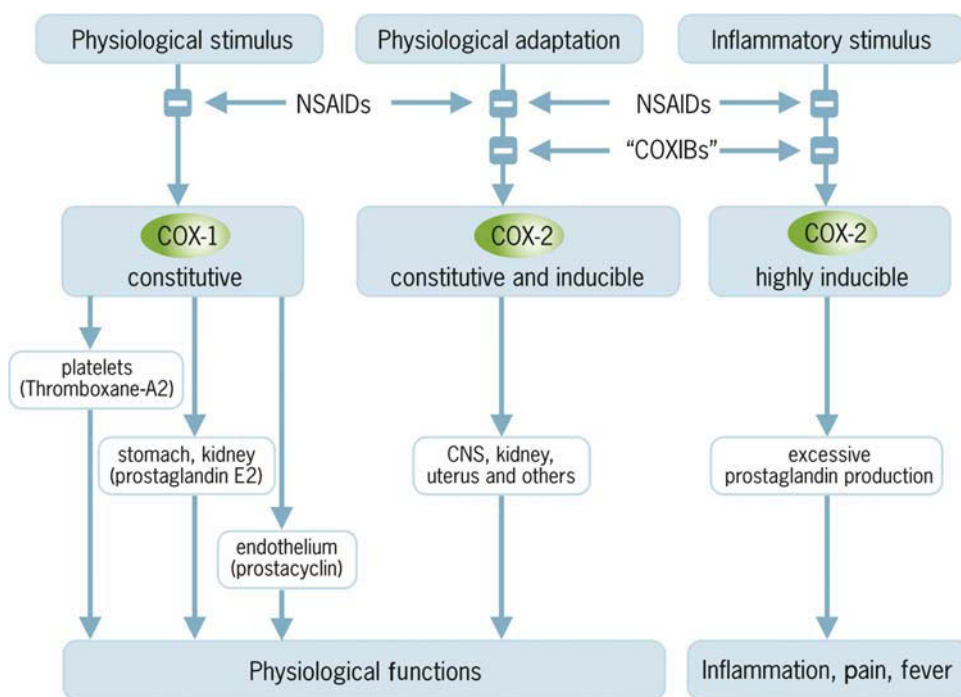
The identification of two distinct types of COX in 1990 [3] encouraged the search for NSAIDs devoid of the side effects associated with ► **COX-1** inhibition. The COX isoform COX-1 is physiologically expressed in the stomach, platelets and the kidney and is therefore responsible for the synthesis of prostaglandins needed for normal organ function (Fig. 2). Its inhibition by conventional NSAIDs causes side effects, e.g. inhibition of prostaglandin synthesis in the gastrointestinal tract results in a loss of protection in the gastrointestinal mucosa and ulcerations. The COX isoform, COX-2 is rapidly induced by various factors including cytokines, and its expression is triggered by inflammation, pain or tissue damage. It is clear from this division of COX into COX-1 and an inducible COX-2 that the anti-inflammatory, analgesic and anti-pyretic effects of the NSAIDs are mainly attributable to inhibition of COX-2 whereas inhibition of COX-1 is associated with some of the unwanted effects of the NSAIDs (Fig. 2).

It follows that drugs that selectively inhibit COX-2 should cause fewer side effects than those that inhibit both COX-1 and COX-2. At therapeutic doses, all currently available NSAIDs, with the exception of celecoxib, etoricoxib, lumiracoxib and parecoxib (the prodrug of valdecoxib), are non-selective and inhibit both COX isoforms.

Newer research has shown that the assignment of physiological activity exclusively to COX-1 and pathological activity exclusively to COX-2 is not strictly valid since COX-2 is expressed constitutively in organs such as spinal cord, kidney or uterus (Fig. 2). Furthermore, COX-2 is formed during various



Non-steroidal Anti-inflammatory Drugs. Figure 1 Non-steroidal anti-inflammatory drugs (NSAIDs) block cyclooxygenase (COX), an enzyme catalysing the production of prostaglandins. When fewer prostaglandins are produced, their physiological and pathophysiological effects are decreased, resulting in therapeutic as well as the unwanted effects of the NSAIDs.



Non-steroidal Anti-inflammatory Drugs. Figure 2 Physiological and pathophysiological functions of the COX isoenzymes COX-1 and COX-2, and the consequences of their inhibition by either unselective conventional NSAIDs or COX-2-selective "COXIBs".

physiological adaptation processes such as the healing of wounds and ulcers.

Clinical Use (Including Side Effects)

NSAIDs are indicated in the treatment of:

- Various pain states (e.g. headache, toothache and migraine), primarily pathophysiological inflammatory pain, e.g. rheumatic pain and pain caused by bone metastases

- Defects of the Ductus arteriosus Botalli (short circuit connection between arteria pulmonalis and aorta; non-closure after birth)
- Fever

Unwanted reactions of NSAIDs include:

- Gastrointestinal disorders (e.g. dyspepsia), gastrointestinal erosion with bleeding, ulceration and perforation

- Kidney malfunctions with retention of sodium and water
- Inhibition of platelet aggregation
- Central nervous symptoms such as dizziness and headache
- Disturbance of uterine motility
- Skin reactions
- Triggering of asthma attacks in asthmatics. This side effect is a pseudo-allergic reaction where COX-inhibition increases the availability of substrates for lipoxygenase, which are converted to broncho-constrictive leukotriens
- Thromboembolic events (myocardial infarction and stroke, see below)

Non-selective inhibitors of prostaglandin synthesis are contraindicated:

- In gastric and duodenal ulcer
- In asthma
- In bleeding disorders
- During the last few weeks of pregnancy because of the danger in the early seal of the Ductus Botalli

Glucocorticoids increase the risk of gastrointestinal complications caused by NSAIDs. Considerable caution is necessary when using NSAIDs in patients with severe liver and kidney damage and they should not be combined with coumarines. Owing to the limited experience obtained, these precautions and contraindications also apply to COX-2-selective inhibitors.

The following drug interactions are the most important that can occur when conventional NSAIDs are co-administered with other agents:

- The uricosuric effect of probenecid is reduced
- The diuretic effect of saluretics is weakened
- The blood glucose-lowering effect of oral antidiabetics is increased
- The elimination of methotrexate is delayed and its toxicity is increased
- The elimination of lithium ions is delayed
- The anti-coagulation effect of coumarin derivatives is enhanced
- The antihypertensive effect of ACE inhibitors is reduced

Due to the short period of clinical use, the interaction profile of COX-2-selective inhibitors cannot be entirely described at the present time.

Derivatives of Salicylic Acid

Salicylic acid for systemic use has been replaced by acetylsalicylic acid, amides of salicylic acid (salicylamide, ethenzamide, salacetamide), salsalate and diflunisal.

Acetylsalicylic Acid (Aspirin)

The esterification of the phenolic hydroxyl group in salicylic acid with acetic acid results not only in an

agent with improved local tolerability but also greater anti-pyretic and anti-inflammatory activity and, in particular, more marked inhibitory effects on platelet aggregation (inhibition of thromboxane-A₂ synthesis). Because of these qualities, acetylsalicylic acid is one of the most frequently used non-opioid analgesics, and the most important inhibitor of platelet aggregation.

Acetylsalicylic acid irreversibly inhibits both COX-1 and COX-2 by acetylating the enzymes. Since mature platelets lack a nucleus, they are unable to synthesise new enzyme. The anti-platelet effects of acetylsalicylic acid persist therefore throughout the lifetime of the platelet and the half-life of this effect is thus being much longer than the elimination half-life of acetylsalicylic acid (15 min). Since new platelets are continuously launched into the circulation, the clinically relevant anti-platelet effect of aspirin lasts for up to five days. This is the reason why low doses of acetylsalicylic acid (ca. 100 mg per day) are sufficient in the prophylaxis of heart attacks.

After oral administration, acetylsalicylic acid is rapidly and almost completely absorbed but in the intestinal mucosa it is partly deacetylated to salicylic acid, which also exhibits analgesic activity. The plasma half-life of acetylsalicylic acid is ~15 min whereas that of salicylic acid, at low dosages of acetylsalicylic acid, is 2–3 h. Salicylic acid is eliminated more slowly when acetylsalicylic acid is administered at high dose rates because of saturation of the liver enzymes. The metabolites are mainly excreted via the kidney.

The dosage of acetylsalicylic acid in the treatment of pain and fever is 1.5–3 g daily and in the prophylaxis of heart attacks 30–100 mg daily.

Side effects of acetylsalicylic acid administration include buzzing in the ears, loss of hearing, dizziness, nausea, vomiting, and most importantly gastrointestinal bleeding, gastrointestinal ulcerations including gastric perforation. The administration of acetylsalicylic acid in children with viral infections can, in rare cases, produce **Reye's syndrome** involving liver damage, encephalopathy and a mortality rate exceeding 50%. Acute salicylate poisoning results in hyperventilation, marked sweating and irritability followed by respiratory paralysis, unconsciousness, hyperthermia and dehydration.

Derivatives of Acetic Acid

Indomethacin

Indomethacin is a strong inhibitor of both COX isoforms with a slight stronger effect in the case of COX-1. It is rapidly and almost completely absorbed from the gastrointestinal tract and has high plasma protein binding (>95%). The plasma half-life of indomethacin varies from 3 to 11 h due to intense enterohepatic cycling. Only about 15% of the substance is eliminated unchanged in the urine, the remainder being eliminated in urine and bile as inactive metabolites (O-demethylation, glucuronidation, N-deacylation).

The daily oral dose of indomethacin is 50–150 mg (up to 200 mg).

Indomethacin treatment is associated with a high incidence (30%) of side effects typical for those seen with other NSAIDs (see above). Gastrointestinal side effects, in particular, are more frequently observed after indomethacin than after administration of other NSAIDs. The market share of indomethacin (~5%) is therefore low compared to that for other non-steroidal anti-rheumatic agents.

Diclofenac

Diclofenac is an exceedingly potent COX inhibitor slightly more efficacious against COX-2 than COX-1. Its absorption from the gastrointestinal tract varies according to the type of pharmaceutical formulation used. The oral bioavailability is only 30–80% due to a first-pass effect. Diclofenac is rapidly metabolised (hydroxylation and conjugation) and has a plasma half-life of 1.5 h. The metabolites are excreted renally and via the bile.

Epidemiological studies have demonstrated that diclofenac causes less serious gastrointestinal complications than indomethacin. However, a rise in plasma liver enzymes occurs more frequently with diclofenac than with other NSAIDs.

The daily oral dose of diclofenac is 50–150 mg. Diclofenac is also available as eye-drops for the treatment of non-specific inflammation of the eye and for the local therapy of eye pain.

Derivatives of Arylpropionic Acids

2-Arylpropionic acid derivatives possess an asymmetrical carbon atom giving rise to S- and R-enantiomers. The S-enantiomer inhibits COX 2–3 times more strongly than the corresponding R-enantiomer. This finding has led to the marketing of pure S-enantiomers (e.g. S-ibuprofen and S-ketoprofen) in some countries in addition to the racemates where the R-enantiomer is considered as “ballast”. However, it is not yet proven whether 2-arylpropionic acids are better tolerated when given as S-enantiomer than as the racemate. Naproxen, for example, which is clinically available only as the S-enantiomer, does not cause less serious gastrointestinal side effects than, e.g. ibuprofen racemate.

Ibuprofen is the most thoroughly researched 2-arylpropionic acid. It is a relatively weak, non-selective inhibitor of COX. In epidemiological studies, ibuprofen compared to all other conventional NSAIDs, has the lowest relative risk of causing severe gastrointestinal side effects. Because of this, ibuprofen is the most frequently used OTC (“over the counter”, sale available without prescription) analgesic. Ibuprofen is highly bound to plasma proteins and has a relatively short elimination half-life (~2 h). It is mainly glucuronidated to inactive metabolites that are eliminated via the kidney.

The typical single oral dose of ibuprofen as an OTC analgesic is 200–400 mg and 400–800 mg when used in

anti-rheumatic therapy. The corresponding maximum daily doses are 1200 or 2400 mg, respectively but the dose in anti-rheumatic therapy in some countries can be as high as 3200 mg daily.

Other arylpropionic acids include naproxen, ketoprofen and flurbiprofen. They share most of the properties of ibuprofen. The daily oral dose of ketoprofen is 50–150 mg, 150–200 mg for flurbiprofen and 250–1000 mg for naproxen. Whereas the plasma elimination half-life of ketoprofen and flurbiprofen are similar to that of ibuprofen (1.5–2.5 h and 2.4–4 h, respectively), naproxen is eliminated much more slowly with a half-life of 13–15 h.

Oxicams

Oxicams, e.g. piroxicam, tenoxicam, meloxicam and lornoxicam are non-specific inhibitors of COX. Like diclofenac, meloxicam inhibits COX-2 ten times more potently than COX-1. This property can be exploited clinically with doses up to 7.5 mg per day, but at higher doses COX-1-inhibition becomes clinically relevant. Since the dose of meloxicam commonly used is 15 mg daily, this agent cannot be regarded as a COX-2-selective NSAID and considerable caution needs to be exercised when making comparisons between the actions of meloxicam and those of other conventional NSAIDs. The average daily dose in anti-rheumatic therapy is 20 mg for piroxicam and tenoxicam, 7.5–15 mg for meloxicam and 12–16 mg for lornoxicam. Oxicams have long elimination half-lives (lornoxicam 3–5 h, meloxicam ~20 h, piroxicam ~40 h and tenoxicam ~70 h).

COX-2-Selective NSAIDs (COXIBs)

The development of the COXIBs has been based on the hypothesis COX-1 is the physiological COX and COX-2, the pathophysiological isoenzyme. Inhibition of the pathophysiological COX-2 only is assumed to result in fewer side effects as compared to non-selective inhibition of both COX isoenzymes (Fig. 2). Celecoxib, etoricoxib and lumiracoxib (in some countries also parecoxib) are the only COXIBs currently approved.

The indications for these agents are in principle identical to those of the non-selective NSAIDs although the substances have not yet received approval for the whole spectrum of indications of the conventional NSAIDs. Because they lack COX-1-inhibiting properties, COX-2-selective inhibitors show fewer side effects than conventional NSAIDs. However, they are not free of side effects because COX-2 has physiological functions that are blocked by the COX-2 inhibitors. The most frequently observed side effects are infections of the upper respiratory tract, diarrhoea, dyspepsia, abdominal discomfort and headache. Peripheral oedema is as frequent as with conventional NSAIDs. The frequency of gastrointestinal complications is approximately half that observed with conventional NSAIDs.

The precise side effect profile of the selective COX-2 inhibitors however, will only be known after several years of clinical use.

Cardiovascular Effects of COXIBs and Non-Selective NSAIDs

Randomised clinical trials and observational studies have clearly shown an increased risk of thromboembolic events during treatment with COXIBs [4]. The incidence of these events tends to increase with the administered COXIB dose and with duration of treatment (for review see [5]). Confusion exists about the underlying mechanisms. Surely the imbalance between the antithrombotic prostacyclin, which is inhibited by COXIBs and the prothrombotic thromboxane-A₂, which is not inhibited by COXIBs due to the COX-2-selectivity of these drugs, plays an important role. However, recent observational studies with non-selective NSAIDs have also shown an increased risk for cardiovascular events with this group of drugs. Non-selective NSAIDs normally do not cause an imbalance between prostacyclin and thromboxane-A₂ since prostaglandins are inhibited unselectively. Thus other mechanisms, both COX-2-dependent and independent ones may play a role for the cardiovascular risk of selective and non-selective NSAIDs (for review see [5]). Unfortunately long-term placebo and active-comparator-controlled trials are nearly lacking for the non-selective NSAIDs. The actual discussion, which is still not finalised, suggests that both COXIBs and non-selective NSAIDs have a similar increased risk for thromboembolic events. The labelling of all drugs has been changed accordingly although the wording differs from country to country. Irrespective from the actual wording, case caution is always necessary when COX-2-selective or non-selective NSAIDs are used.

- Analgesics
- Inflammation
- Nociception
- Opioid Systems

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Non-steroidal Anti-rheumatic Drugs

► Non-steroidal Anti-inflammatory Drugs

Non-viral Peptidases

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Synonyms

Proteases; Proteinases; Proteolytic enzymes

Definition

Peptidases are enzymes that catalyse the hydrolysis of peptide bonds – the bonds between amino acids that are found in peptides and proteins. The terms “protease”, “proteinase” and “proteolytic enzyme” are synonymous, but strictly speaking can only be applied to peptidases that hydrolyse bonds in proteins. Because there are many peptidases that act only on peptides, the term “peptidase” is recommended. Peptidases are included in subclass 3.4 of enzyme nomenclature [1,5].

Basic Characteristics

Active Site Residues

Peptidases are found in all forms of life. There are about 612 peptidases in humans, plus an additional 385 proteins that are homologous to peptidases but either have no or different catalytic activity. In general, 2% of all genes in a genome encode peptidases.

Cleavage of a peptide bond is an example of a nucleophilic attack. The nucleophile in the reaction is either an activated water molecule or part of the side-chain of an amino acid, and peptidases are described as having either a ►water nucleophile or a ►protein nucleophile. Peptidases with a water nucleophile either utilize one or two metal ions as ligands for the water molecule, in which case the peptidase generally acts

at neutral pH, or the water is bound by two aspartic acid residues, in which case the peptidase usually has an acidic pH optimum. For peptidases with a protein nucleophile, the nucleophile is either the hydroxyl group from a serine or threonine residue or the thiol group from cysteine. Thus there are five major catalytic types: aspartic, metallo, serine, threonine and cysteine peptidases. A sixth type, glutamic peptidases, is known, but the only known example is from fungi. There are still several peptidases for which the catalytic type is as yet undetermined.

A metallopeptidase binds a divalent metal ion tetrahedrally, with three ligands being amino acid residues and the fourth the water molecule that becomes the nucleophile in the reaction. The amino acid ligand residues are conserved. Most commonly, a zinc ion is bound, but peptidases that bind cobalt, manganese, nickel and copper also exist. Metal ligands are most often histidine residues, but can also be aspartic and glutamic acids, and more unusually asparagine, glutamine or lysine. When a peptidase binds two metal ions five residues are involved, one of which ligates both ions. The metal ion is described as ►co-catalytic. Besides metal ligands other residues are often essential for catalytic activity. The peptidases that are involved in the turnover of extracellular matrices, known as ►matrixins or ►matrix-metallopeptidases (MMPs) are examples of metallopeptidases containing the motif HEXXH. In this motif the two histidines are metal ligands while the glutamic acid is an ►active site residue. Glutamate carboxypeptidase binds two zinc ions via two histidines, an aspartic acid and two glutamic acids and in addition has two active site residues, an aspartic and a glutamic acid.

Aspartic peptidases bind and activate water via two aspartic acid residues.

Peptidases with a protein nucleophile often have an active site containing two or three residues. A second residue, most often a histidine (though lysine and arginine can substitute), is required to interact with the nucleophile and become a ►protor acceptor or ►general base in the catalytic reaction. If a ►catalytic triad exists, which is often the case when a histidine residue is the general base, then an aspartic or glutamic acid, asparagine or glutamine is thought to orientate the imidazolium ring of the histidine side chain. In the cysteine peptidase cathepsin B, the order of the catalytic residues is Cys, His, Asn. In the serine peptidase chymotrypsin, the order is His, Asp, Ser.

For many serine and cysteine peptidases catalysis first involves formation of a complex known as an ►acyl intermediate. An essential residue is required to stabilize this intermediate by helping to form the ►oxyanion hole. In cathepsin B a glutamine performs this role and sometimes a catalytic tetrad (Gln, Cys, His, Asn) is referred to. In chymotrypsin, a glycine is essential for stabilizing the oxyanion hole.

Threonine peptidases (and some cysteine and serine peptidases) have only one active site residue, which is the N-terminus of the mature protein. Such a peptidase is known as an ►N-terminal nucleophile hydrolase or ►Ntn-hydrolase. The amino group of the N-terminal residue performs the role of the general base. The catalytic subunits of the proteasome are examples of Ntn-hydrolases.

The order and nature of the active site residues and metal ligands is conserved between homologous peptidases. All the members of a family will have the same catalytic type.

Classification and Nomenclature

Peptidases have been classified by the *MEROPS* system since 1993 [2], which has been available via the *MEROPS* database since 1996 [3]. The classification is based on sequence and structural similarities. Because peptidases are often multidomain proteins, only the domain directly involved in catalysis, and which bears the active site residues, is used in comparisons. This domain is known as the ►peptidase unit. Peptidases with statistically significant peptidase unit sequence similarities are included in the same ►family. To date 186 families of peptidase have been detected. Examples from 86 of these families are known in humans. A family is named from a letter representing the catalytic type (“A” for aspartic, “G” for glutamic, “M” for metallo, “C” for cysteine, “S” for serine and “T” for threonine) plus a number. Examples of family names are shown in Table 1. There are 53 families of metallopeptidases (24 in human), 14 of aspartic peptidases (three of which are found in human), 62 of cysteine peptidases (19 in human), 42 of serine peptidases (17 in human), four of threonine peptidases (three in human), one of glutamic peptidases and nine families for which the catalytic type is unknown (one in human). It should be noted that within a family not all of the members will be peptidases. Usually ►non-peptidase homologues are a minority and can be easily detected because not all of the active site residues are conserved.

All peptidases within a family will have a similar tertiary structure, and it is not uncommon for peptidases in one family to have a similar structure to peptidases in another family, even though there is no significant sequence similarity. Families of peptidases with similar structures and the same order of active site residues are included in the same ►clan. A clan name consists of two letters, the first representing the catalytic type as before, but with the extra letter “P”, and the second assigned sequentially. Unlike families, a clan may contain peptidases of more than one catalytic type. So far this has only been seen for peptidases with protein nucleophiles, and these clans are named with an initial “P”. Only three such clans are known. Clan PA includes peptidases with a chymotrypsin-like fold, which besides serine peptidases such as chymotrypsin

Non-viral Peptidases. Table 1 Pathological conditions involving peptidases

Clan	Family	Code	Peptidase	Pathology or pharmacology
AA	A1	A01.001	Pepsin A	Elevated in gastric ulcer
AA	A1	A01.003	Gastricsin	Polymorphism correlates with gastric ulceration
AA	A1	A01.004	Memapsin-2	Potential drug target for Alzheimer's disease
AA	A1	A01.007	Renin	Potential drug target for hypertension
AA	A1	A01.009	Cathepsin D	Diagnosis and prognosis of breast and other cancers
AA	A1	A01.010	Cathepsin E	Excitotoxin-induced neuronal cell death
AA	A1	A01.014	Candidapepsin SAP1	Epithelial damage in vaginal candidiasis
AA	A1	A01.022	Plasmepsin-1	Potential drug target for malaria
AA	A1	A01.023	Plasmepsin-2	Potential drug target for malaria
AA	A1	A01.037	Canditropsin	Possible drug target for candidiasis
AA	A1	A01.041	Memapsin-1	Candidate beta-secretase in Alzheimer's disease
AA	A1	A01.046	Napsin A	Diagnosis of primary lung adenocarcinoma
AA	A1	A01.060	Candidapepsin SAP2	Epithelial damage in vaginal candidiasis
AC	A8	A08.001	Signal peptidase II	Drug target for <i>Mycobacterium tuberculosis</i> infection
AD	A22	A22.001	Presenilin 1	Gamma-secretase of Alzheimer's disease, and a drug target
CA	C1	C01.002	Chymopapain	Treatment of herniated intervertebral disk
CA	C1	C01.005	Stem bromelain	Burn debridement
CA	C1	C01.006	Ficain	Anthelmintic
CA	C1	C01.009	Cathepsin V	Immunopathogenesis of myasthenia gravis
CA	C1	C01.026	Ananain	Burn debridement
CA	C1	C01.032	Cathepsin L	
CA	C1	C01.033	Cathepsin L-like peptidase (<i>Fasciola</i> sp.)	Potential drug target for liver fluke infection
CA	C1	C01.034	Cathepsin S	Tissue damage associated with inflammation
CA	C1	C01.036	Cathepsin K	Potential drug target for control of osteoporosis
CA	C1	C01.040	Cathepsin H	
CA	C1	C01.046	Falcipain-2	Potential drug target for malaria infection
CA	C1	C01.050	Histolysain	Potential drug target for amoebic dysentery
CA	C1	C01.060	Cathepsin B	Potential drug target for cancer
CA	C1	C01.070	Dipeptidyl-peptidase I	Potential therapeutic target for treatment of sepsis
CA	C1	C01.073	Peptidase 1 (mite)	Potential drug target for asthma
CA	C1	C01.075	Cruzipain	Potential drug target for Chagas disease
CA	C1	C01.076	CPA peptidase	Potential drug target for leishmaniasis
CA	C1	C01.077	Falcipain-1	Potential drug target for malaria.
CA	C1	C01.079	Papain homologue (<i>Theileria</i> -type)	Potential drug target for East coast fever and theileriosis
CA	C1	C01.084	Bleomycin hydrolase (animal)	Destroys chemotherapeutic bleomycin
CA	C1	C01.094	Giardain	Potential drug target for <i>Giardia</i> parasitism
CA	C2	C02.001	Calpain-1	Drug target for stroke and neural injuries
CA	C2	C02.002	Calpain-2	Drug target for stroke and neural injuries
CA	C19	C19.001	Isopeptidase T	
CD	C13	C13.004	Legumain	
CD	C14	C14.001	Caspase-1	Potential drug target for down-regulation of the inflammatory mediator, interleukin 1beta, which could ameliorate inflammation and endotoxic shock
CD	C14	C14.003	Caspase-3	Potential drug target for cardiac ischemic injury
CD	C14	C14.004	Caspase-7	Potential drug target for cardiac ischemic injury
CD	C14	C14.005	Caspase-6	Tumour-cell-killing
CD	C14	C14.010	Caspase-9	Potential drug target for amyotrophic lateral sclerosis

Non-viral Peptidases. Table 1 Pathological conditions involving peptidases (Continued)

Clan	Family	Code	Peptidase	Pathology or pharmacology
CD	C14	C14.012	Caspase-11	Potential drug target in lipopolysaccharide-induced septic shock
CD	C25	C25.001	Gingipain R	Possible drug target in periodontitis
CD	C25	C25.002	Gingipain K	Possible drug target in periodontitis
CF	C15	C15.010	Pyroglutamyl-peptidase I (chordate)	
MA	M1	M01.001	Aminopeptidase N	Drug target for analgesia
MA	M1	M01.003	Aminopeptidase A	Drug target for hypertension and angiogenesis in cancer
MA	M1	M01.004	Leukotriene A4 hydrolase	Drug target to control inflammation
MA	M2	M02.001	Angiotensin-converting enzyme peptidase unit 1	Drug target for the control of hypertension
MA	M2	M02.004	Angiotensin-converting enzyme peptidase unit 2	Drug target for the control of hypertension
MA	M3	M03.001	Thimet oligopeptidase	
MA	M3	M03.006	Mitochondrial intermediate peptidase	
MA	M8	M08.001	Leishmanolysin	Target for vaccine production
MA	M9	M09.001	Bacterial collagenase (<i>Vibrio</i> -type)	Potential agent for burn debridement
MA	M9	M09.002	Collagenase colA	Treatment of bed sores
MA	M10	M10.001	Matrix metallopeptidase-1	Drug target for prevention of pathological tissue damage
MA	M10	M10.002	Matrix metallopeptidase-8	Drug target for prevention of pathological tissue damage
MA	M10	M10.003	Matrix metallopeptidase-2	Drug target for prevention of pathological tissue damage
MA	M10	M10.004	Matrix metallopeptidase-9	Drug target for prevention of pathological tissue damage
MA	M10	M10.005	Matrix metallopeptidase-3	Drug target for prevention of pathological tissue damage
MA	M10	M10.014	Membrane-type matrix metallopeptidase-1	Potential drug target for tumor cell invasion
MA	M10	M10.056	Aeruginolysin	Target for vaccine development, and chemotherapy of bacterial infection
MA	M12	M12.005	Procollagen C-peptidase	Drug target for fibrosis
MA	M12	M12.133	Fibrolase (<i>Agkistrodon contortrix</i>)	Potential target for vaccine development.
MA	M12	M12.147	Atroxase	Potential anticoagulant or thrombolytic agent.
MA	M12	M12.210	ADAM10 peptidase	Potential drug target for demyelinating disease
MA	M12	M12.217	ADAM17 peptidase	Potential drug target for control of formation of tumor necrosis factor alpha
MA	M12	M12.221	ADAMTS4 peptidase	Potential drug target for the treatment of osteoarthritis
MA	M12	M12.225	ADAMTS5 peptidase	Potential drug target for the treatment of osteoarthritis
MA	M13	M13.001	Neprilysin	Drug target in hypertension and renal disease
MA	M13	M13.002	Endothelin-converting enzyme 1	Drug target in hypertension.
MA	M27	M27.001	Tentoxilysin	Essential for action of tetanus toxin
MA	M27	M27.002	Bontoxilysin	Therapeutic use for local paralysis of neuromuscular function, as in strabismus
MA	M34	M34.001	Anthrax lethal factor	Component of anthrax toxin
MA	M41	M41.006	Paraplegin	Potential therapeutic agent for patients with paraplegin deficiency
MC	M14	M14.009	Carboxypeptidase U	Potential use in antithrombotic and thrombolytic therapy
MD	M15	M15.010	vanY D-Ala- D-Ala carboxypeptidase	Target for antibacterial drugs
MD	M15	M15.011	vanX D-Ala- D-Ala dipeptidase	Target for antibacterial drugs

Non-viral Peptidases. Table 1 Pathological conditions involving peptidases (Continued)

Clan	Family	Code	Peptidase	Pathology or pharmacology
MG	M24	M24.002	Methionyl aminopeptidase 2	Target of the potent angiogenesis inhibitors fumagillin and ovalicin
MH	M20	M20.001	Glutamate carboxypeptidase	Rescue agent during high-dose methotrexate therapy
MH	M20	M20.006	Carnosine dipeptidase I	Deficiency leads to homocarnosinosis
MH	M28	M28.010	Glutamate carboxypeptidase II	Diagnosis and assessment of prostatic carcinoma
MJ	M19	M19.001	Membrane dipeptidase	Target for cilastatin
MO	M23	M23.002	Staphylolysin	Potential use for the treatment of keratitis
MO	M23	M23.004	Lysostaphin	Use in treatment of endophthalmitis mediated by methicillin-resistant <i>Staphylococcus aureus</i>
PA	S1	S01.001	Chymotrypsin A	
PA	S1	S01.010	Granzyme B (human-type)	
PA	S1	S01.123	Granzyme B (rodent-type)	
PA	S1	S01.131	Neutrophil elastase	Drug target for emphysema, cystic fibrosis, adult respiratory distress syndrome, rheumatoid arthritis and other diseases
PA	S1	S01.134	Myeloblastin	Antigen against which the autoimmunity of Wegener's granulomatosis is directed
PA	S1	S01.140	Chymase (human-type)	Possible drug target in immune-related diseases of lung
PA	S1	S01.143	Tryptase alpha	Drug target for asthma and antigen-induced bronchoconstriction
PA	S1	S01.151	Trypsin I	
PA	S1	S01.153	Pancreatic elastase	Drug target for acute pancreatitis
PA	S1	S01.155	Pancreatic elastase II	Drug target for acute pancreatitis
PA	S1	S01.156	Enteropeptidase	Potential drug target for acute pancreatitis
PA	S1	S01.162	Kallikrein hK3	Potential drug target for prostatic and other cancers
PA	S1	S01.176	Batroxobin	Defibrinating agent
PA	S1	S01.177	Crotalase	Defibrinating agent
PA	S1	S01.178	Ancrod	Defibrinating agent
PA	S1	S01.214	Coagulation factor IXa	Possible target for gene therapy of haemophilia B
PA	S1	S01.215	Coagulation factor VIIa	Drug target for control of coagulation
PA	S1	S01.216	Coagulation factor Xa	Drug target for control of coagulation
PA	S1	S01.217	Thrombin	Drug target for thrombosis
PA	S1	S01.232	t-Plasminogen activator	Used as therapeutic thrombolytic agent
PA	S1	S01.233	Plasmin	
PA	S1	S01.244	Neuropsin	Potential drug target for epilepsy
PA	S1	S01.251	Prostase	Proposed for use in vaccine strategies against prostate cancer
PA	S1	S01.258	Trypsin-2 (human-type)	Potential use in detection of acute pancreatitis
PA	S1	S01.270	Exfoliatin A	Drug target in staphylococcal scalded skin syndrome
PA	S1	S01.281	Arginyl peptidase	Potential drug target in corneal infection by <i>Pseudomonas aeruginosa</i>
PA	S1	S01.302	Matriptase	Drug target for some forms of cancer
PA	S6	S06.002	EspP g.p. (<i>Escherichia coli</i>)	Potential virulence factor that cleaves human coagulation factor V
PA	S6	S06.003	Tsh peptidase (<i>Escherichia coli</i>)	Potential virulence factor that cleaves human coagulation factor V
PB	T1	T01.010	Proteasome catalytic subunit 1	Potential use in cancer, rheumatoid arthritis and psoriasis that are characterized by these processes
PB	T1	T01.011	Proteasome catalytic subunit 2	Potential use in cancer, rheumatoid arthritis and psoriasis that are characterized by these processes

Non-viral Peptidases. Table 1 Pathological conditions involving peptidases (Continued)

Clan	Family	Code	Peptidase	Pathology or pharmacology
PB	T1	T01.012	Proteasome catalytic subunit 3	Potential use in cancer, rheumatoid arthritis and psoriasis that are characterized by these processes
PC	C26	C26.001	Gamma-glutamyl hydrolase	
PC	C56	C56.002	DJ-1 putative peptidase	Mutations in the gene cause PARK7, an autosomal recessive form of early-onset parkinsonism
PC	S51	S51.002	Alpha-aspartyl dipeptidase	
SB	S8	S08.020	C5a peptidase	Proposed target for vaccine against group B streptococcal infection
SB	S8	S08.090	Tripeptidyl-peptidase II	Drug target because of cholecystokinin-8 degradation
SC	S9	S09.001	Prolyl oligopeptidase	Potential drug target
SC	S9	S09.003	Dipeptidyl-peptidase IV (eukaryote)	Drug target for type II diabetes
SC	S9	S09.004	Acylaminoacyl-peptidase	
SF	S26	S26.001	Signal peptidase I	Potential drug target for antibacterial agents
SF	S26	S26.009	Signalase (eukaryote) 18 kDa component	Potential drug target to suppress human coronavirus replication
SF	S26	S26.010	Signalase (eukaryote) 21 kDa component	Potential drug target to suppress human coronavirus replication

Links are provided to the relevant summary pages in the *MEROPS* database.

also includes some cysteine peptidases from viruses. Clan PB contains the Ntn-hydrolases, which can be threonine, cysteine or serine in type. Clan PC includes the cysteine peptidase gamma-glutamyl hydrolase and the serine peptidase alpha-aspartyl dipeptidase. There are 45 clans of peptidases, or in other words there are 45 independent origins of peptidases.

Peptidases that are thought to be species variants of the same protein and have similar properties are included in the same [►MEROPS identifier](#). The identifier consists of the three-character family name (padded with zeroes if necessary), a dot, and a unique three digit number. Examples of *MEROPS* identifiers are shown in Table 1. Two special kinds of identifier are used for non-peptidase homologues in the *MEROPS* database for proteins and pseudogenes. For proteins the three digit number begins with a nine, and for pseudogenes the first digit is replaced by a “P”.

With the onset of genomic biology, there are now many sequences derived from genome sequencing projects that are too divergent to be considered species variants of known peptidases. Of the 54,124 sequences in the *MEROPS* database only 18,741 (34.6%) have been assigned to an identifier.

It is recommended that a well-characterized peptidase should have a trivial name. Although not rigidly adhered to, there is a different suffix for each catalytic type, metallopeptidases names end with “lysin”, aspartic peptidases with “pepsin”, cysteine peptidase with “ain” and serine peptidases with “in”.

Compound and Complex Peptidases

Most peptidases have only one peptidase unit; however, there are some exceptions. A [►compound peptidase](#) has more than one peptidase unit within a single protein molecule. An example is the somatic form of the angiotensin-converting enzyme, which has two peptidase units. The testicular form of this enzyme contains only the second peptidase unit and is transcribed from the same gene but at an alternative initiating methionine. Each peptidase unit has a *MEROPS* identifier, but additionally there is a special identifier for each compound peptidase, which is XM02-001 in the case of angiotensin-converting enzyme.

A [►complex peptidase](#) consists of several proteins in a complex. The 20S proteasome is a complex of 28 subunits, arranged in four stacked rings each containing 7 different subunits. The first and fourth rings contain the same seven (alpha) subunits, as do the second and third (beta subunits). Each subunit is the product of an individual gene, and the subunits are homologous to each other. Only three of the beta subunits are catalytically active, each conferring a different specificity (trypsin-like, chymotrypsin-like or peptidylglutamyl peptide hydrolyzing, now often known as caspase-like). The proteins form a cylinder with the six active sites on the inside and the substrate protein is threaded through one end and degraded to peptides that exit at the opposite end. The 28S proteasome contains additional subunits which form a cap and attach to one end of the 20S proteasome. These

extra subunits are important for unfolding the substrate protein. Again, there is not only a *MEROPS* identifier for each alpha and beta subunit, but also identifiers for the complexes. The 20S proteasome is XT01-001 and the 28S proteasome is XT01-002.

Precursors and Zymogens

Most peptidases are not synthesized as active enzyme but as precursors or ►**zymogens**. The zymogen is transported to the site where it is needed and then activated, usually by the removal of an N-terminal ►**propeptide** by proteolysis. For some members of family S1, especially members of the blood coagulation pathway, a propeptide is not released but cleavage is still required for activity. The propeptide remains attached to the peptidase by a disulfide bridge, becoming the heavy chain in a two-chain complex. Cleavage generates a new hydrophobic N-terminal residue, and a molecular rearrangement then activates the peptidase. For some members of peptidase family M10, the propeptide acts by providing a fourth ligand for the catalytic zinc ion, and only on release of the propeptide does the zinc interact with water and the peptidase become active. Because this fourth ligand is a cysteine, this method of activation is known as a ►**cysteine switch**.

Specificity and Substrate-Binding

The ►**specificity** of a peptidase describes where in a peptide or protein sequence cleavage will occur. Some peptidases will only cleave near the amino- and carboxy-termini of the substrate and these are termed ►**exopeptidases**. A peptidase that does not require free termini and can cleave at any acceptable site within a peptide is termed an ►**endopeptidase**. Most endopeptidases are able to cleave proteins, but there are a few that can only cleave short peptides, and these are known as ►**oligopeptidases**. Neprilysin is an oligopeptidase that degrades peptide hormones such as substance P, thereby switching off the physiological signal. Some endopeptidases are ►**isopeptidases**, cleaving non-standard peptide bonds. An example is isopeptidase T, which releases ubiquitin from ubiquitinated proteins by cleaving the isopeptide bond between the C-terminal Gly of ubiquitin and the amino group of a non-terminal lysine on the protein targeted for degradation.

There are several different types of exopeptidases: ►**aminopeptidases**, ►**carboxypeptidases**, ►**dipeptidyl-peptidases**, ►**tripeptidyl-peptidases**, ►**peptidyl-dipeptidases**, ►**dipeptidases** and ►**omega peptidases**.

Generally, a family of peptidases contains either exopeptidases or endopeptidases, but there are exceptions. Family C1 contains not only endopeptidases such as cathepsin L, but also the aminopeptidase bleomycin hydrolase. Some members of this family can act as exopeptidases as well as endopeptidases. For example, cathepsin B also acts as a peptidyl-dipeptidase, and

cathepsin H also acts as an aminopeptidase. Family S9 includes prolyl oligopeptidase, dipeptidyl-peptidase IV and the omega peptidase acylaminoacyl-peptidase.

The specificity of an endopeptidase is more difficult to describe than that of an exopeptidase. A peptidase with ►**limited specificity** will only cleave after one type of amino acid. Examples are trypsin, which cleaves proteins at lysyl or arginyl bonds, and chymotrypsin, which cleaves after hydrophobic residues. Granzyme B and caspase-3, which are involved in ►**apoptosis** or programmed cell death, cleave only after aspartyl bonds. However, even this can be simplistic; recent evidence has shown that granzyme B from mouse does not cleave Bid, whereas in human cleavage of this protein initiates the apoptotic pathway. The lysosomal endopeptidase legumain cleaves asparaginyl bonds, but under acidic conditions can cleave aspartyl bonds; experiments with processing of the tetanus toxin have shown that only a few asparaginyl bonds are cleaved. Whether this is because only a few bonds are available to the peptidase because of the structure of the substrate or because the specificity of the peptidase is more complicated is unclear. Peptidases that cleave a variety of peptide bonds are described as having ►**broad specificity**.

Cleavage occurs at the ►**scissile bond**. Residues in the substrate towards the N-terminus are numbered P1, P2, P3, etc, whereas residues towards the C-terminus are numbered P1', P2', P3' etc. Cleavage occurs between P1 and P1'. For a peptidase with limited specificity, only the residue in P1 or P1' is important for specificity. A peptidase with an ►**extended substrate binding site** will have a preference for residues in other positions. For example cathepsin L prefers substrates with phenylalanine in P2 and arginine in P1. However, this is a preference only, and cathepsin L cleaves substrates after other amino acids. Caspase-3 has a preference for Asp in both P4 and P1, but it is unusual for substrate specificity to extend much further from the scissile bond. The peptidase with the most extended substrate specificity may be mitochondrial intermediate peptidase that removes an octopeptide targeting signal from the N-terminus of cytoplasmically synthesized proteins that are destined for import into the mitochondrial lumen.

A peptidase has a series of ►**substrate-binding pockets** to accommodate residues of the substrate. The S1 binding pocket accommodates residue P1, the S1' binding pocket accommodates residue P1' and so on. A binding pocket may contain several residues that interact with the substrate, and a single residue may take part in more than one binding pocket. Some peptidases bind the substrate at more than one binding site. A binding site away from the active site is described as an ►**exosite**. The presence of one or more exosites makes the peptidase very specific for a substrate, and it is not unusual for a

peptidase with an exosite to cleave just one protein. Peptidases with such limited specificity include the blood coagulation enzymes. In some peptidases, additional substrate-binding sites are located on domains other than the peptidase unit. For example the matrixins (family M10) interact with proteins of the extracellular matrix through the haemopexin-like domain that is C-terminal to the peptidase unit.

A ►**proteolytic cascade** occurs when one peptidase activates the next in a proteolytic pathway, and this in turn activates the next and so on. This is a mechanism to amplify the initial signal, because one peptidase molecule can activate many zymogen molecules. Examples of proteolytic cascades include blood coagulation, activation of digestive peptidases in the intestine, and apoptosis.

Drugs

The action of a peptidase can be neutralized by an inhibitor. Some inhibitors are very broad in their action and are capable of inhibiting many different peptidases, including peptidases of different catalytic types. Some inhibitors are assumed to be specific for a particular catalytic type, but can inhibit peptidases of different types. Leupeptin, for example, is widely used as an inhibitor of serine peptidases from family S1, but it is also known to inhibit cysteine peptidases from family C1. Cysteine peptidase inhibitors such as iodoacetic acid interact with the thiol of the catalytic cysteine. However, this reduction can occur on any thiol group and can affect other, predominantly intracellular, peptidases with a thiol dependency. One example is thimet oligopeptidase. Metal chelators such as EDTA can inhibit metallopeptidases, but can also affect peptidases that have a requirement for metal ions that is independent of their catalytic activity, such as the calcium-dependent cysteine endopeptidase calpain 1.

Inhibitors which interact only with peptidases of one catalytic type include pepstatin (aspartic peptidases); E64 (cysteine peptidases from clan CA); diisopropyl fluorophosphates (DFP) and phenylmethane sulfonyl-fluoride (PMSF) (serine peptidases). Bestatin is a useful inhibitor of aminopeptidases.

Table 1 lists peptidases that are known or potential drug targets. In nearly all cases the drug will be a synthetic peptidase inhibitor [4].

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Noradrenaline Transporter

Noradrenaline transporters (NAT) are localized in the presynaptic plasma membrane of adrenergic nerve terminals. They belong to a family of proteins with 12 putative transmembrane proteins which are responsible for recycling of released neurotransmitters (noradrenaline/adrenaline, dopamine, serotonin, amino acid transmitters) back into the presynaptic nerve ending. Noradrenaline transporters can be blocked by a number of different antidepressant drugs, including tricyclic antidepressants (e.g. desipramine) and selective noradrenaline reuptake inhibitors (e.g. reboxetine).

- Antidepressants
- Synaptic Transmission

Norepinephrine/Noradrenalin

Neurotransmitter and biogenic amine synthesized by neurons in the locus coeruleus from tyrosine which controls behavioral state, postural tone, selective attention, mood and memory extinction, and is part of sympathoadrenal stress management system.

- α -Adrenergic System
- β -Adrenergic System
- Antidepressant Drugs
- Synaptic Transmission

Normoglycaemia

Euglycaemia, blood glucose concentration within the normal range e.g. fasting blood glucose 3.5 – 6.5 mmol/l; postprandial blood glucose 5 – 11 mmol/l.

- Diabetes Mellitus

NOS

- NO Synthases

NPA Motif

A highly conserved Asn-Pro-Ala (NPA) motif at the narrowest point of the channel that ensures the high selectivity of the pore for water molecules.

- Aquaporins

NPY

- Neuropeptide Y

NPY₃₋₃₆

NPY₃₋₃₆ is an endogenously occurring fragment of *neuropeptide Y*, which is generated upon cleavage by the enzyme *dipeptidylpeptidase IV*, also known as *CD26*. In contrast to *neuropeptide Y*, NPY₃₋₃₆ is selective for the Y₂ and Y₅ relative to the Y₁ subtype of NPY receptors.

- Neuropeptide Y

NSAIDs

- Non-steroidal Anti-inflammatory Drugs

NSAR

- Non-steroidal Anti-inflammatory Drugs

NSF

NSF, the acronym for NEM-sensitive fusion protein, was originally discovered as an essential factor in intracellular membrane transport steps. NSF is now known to catalyze the disassembly of all SNARE complexes. NSF requires the binding of SNAPs to exert its action. NSF is an evolutionarily conserved protein that forms hexameric double-ring structures. During its catalytic cycle, ATP is cleaved and the N-terminal region undergoes massive conformational changes. Recently, NSF has been shown to interact with other proteins such as glutamate receptors, but the significance of these interactions is still controversial.

- Exocytosis

NSMRI

- Non-selective Monoamine Reuptake Inhibitors

Ntn-Hydrolase

An enzyme in which the single catalytic residue is at the N-terminus of the protein. Many Ntn-hydrolases are synthesized as precursors and autoactivate; the precursors are therefore peptidases, even if the mature enzyme has no further proteolytic activity. Three of the beta subunits of the proteasome are Ntn-hydrolases.

- Non-viral Peptidases

Nuclear Factor Kappa B

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Synonyms

Rel proteins

Definition

Nuclear factor kappa B (NF- κ B) is the generic term for a family of dimeric eukaryotic transcription factors, composed of members of the Rel family of DNA-binding proteins including the mammalian proteins RelA (or p65), cRel, RelB, p50 and p52, and the *Drosophila* proteins Dorsal, Dif and Relish. These proteins bind with different affinities to a consensus DNA sequence motif (called the κ B site) consisting of the sequence 5'-GGGRNYYCC-3' in which R is a purine, Y is a pyrimidine, and N is any base.

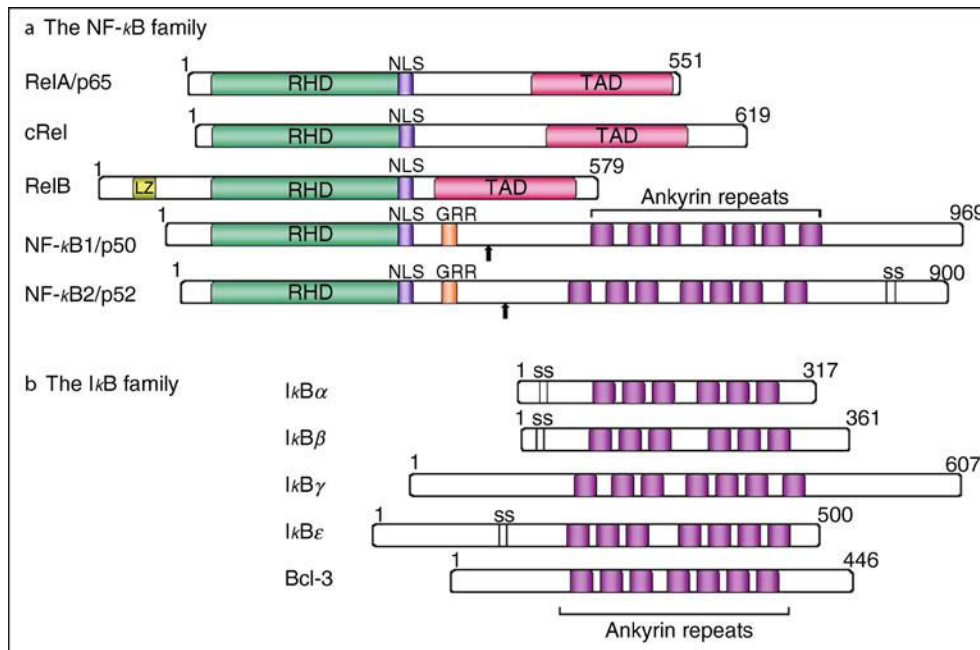
Basic Characteristics

NF- κ B Structure

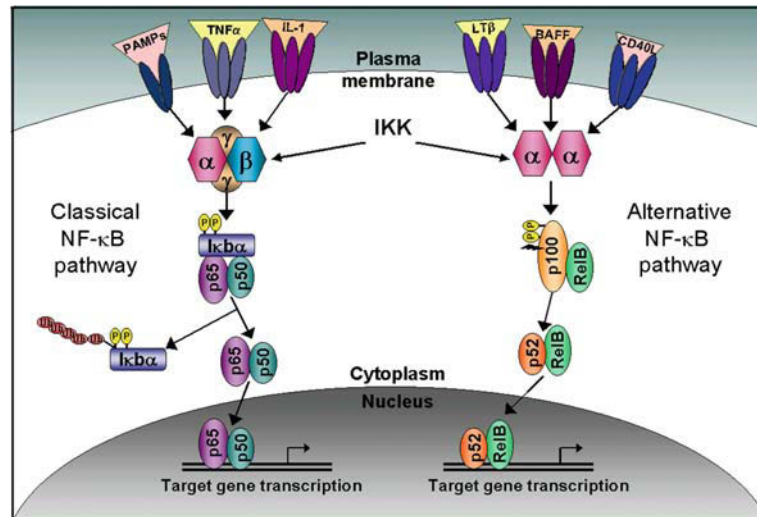
NF- κ B is a dimeric, ubiquitously expressed, and evolutionary conserved transcription factor that plays a critical role in immune and inflammatory responses [1]. In mammals, the NF- κ B family consists of five members: NF- κ B1 (p105/p50), NF- κ B2 (p100/p52), RelA (p65), cRel, and RelB. Structurally, NF- κ B proteins share a highly conserved 300 amino acid

►Rel homology domain (RHD) that encompasses a sequence-specific DNA-binding domain, a dimerization domain and a nuclear translocation signal (NLS) (Fig. 1a). RelA, cRel, and RelB contain a transcription activation domain (TAD). NF- κ B1 and NF- κ B2 are synthesized as large precursors, p105 and p100, that are posttranslationally processed to generate the mature forms, p50 and p52, which lack a TAD.

NF- κ B proteins are principally regulated by subcellular localization whereby the inactive cytoplasmic forms become transcriptionally active proteins upon translocation to the nucleus. In most resting cells, NF- κ B is maintained in the cytoplasm by binding to members of the inhibitor of κ B (I κ B) family (Fig. 1b). The I κ Bs (such as I κ B α , I κ B β , and I κ B ϵ) contain 6–7 ►ankyrin repeats that mediate their binding to the RHD and thereby interfere with the function of the NLS. Ankyrin repeats are also found in the precursors NF- κ B1 (p105) and NF- κ B2 (p100), enabling them to function as I κ Bs and retain Rel proteins in the cytoplasm of unstimulated cells.



Nuclear Factor Kappa B. Figure 1 Mammalian NF- κ B proteins and their regulators, the I κ B proteins. (a) NF- κ B proteins share a highly conserved 300 amino acids Rel homology domain (RHD) located at their amino-terminal region, which includes a DNA-binding domain, a dimerization domain, and a nuclear localization signal (NLS). RelA (p65), cRel, and RelB contain in their carboxy-terminal part, a nonhomologous transcriptional activation domain (TAD). RelB has an additional amino-terminal leucine zipper (LZ) motif. NF- κ B1/p50 (or p105/p50) and NF- κ B2/p52 (or p100/p52) contain a glycine-rich region (GRR) and carboxy-terminal ankyrin repeats that are also present in I κ B proteins. Proteolytic processing of NF- κ B1/p50 and NF- κ B2/p52 (sites indicated by arrows) generates the respective mature NF- κ B proteins, p50, and p52. (b) The inhibitor of NF- κ B (I κ B) family members including I κ B α , I κ B β , I κ B γ , I κ B ϵ , and Bcl-3, are characterized by the presence of multiple ankyrin repeats that are essential for binding of I κ Bs to the RHD of NF- κ B proteins. All three I κ B subunits, I κ B α , I κ B β , and I κ B ϵ present in their amino-terminal region two critical serine residues (depicted SS) required for their inducible phosphorylation-mediated degradation.



Nuclear Factor Kappa B. Figure 2 Classical and alternative NF-κB activation pathways. The classical or canonical NF-κB pathway is activated by a variety of stimuli including the proinflammatory cytokines, TNFα and IL-1, and diverse pathogen-associated molecular patterns (PAMPs). Each signal is transmitted to the classical IKK complex (composed of IKKα, IKKβ, and IKKγ) through activation of specific transmembrane receptors. IKK phosphorylates the IκB proteins leading to their polyubiquitination and degradation. This leads to liberation and nuclear translocation of NF-κB dimers (mainly p65–p50) and coordinated activation of multiple inflammatory and innate immune genes. The alternative or noncanonical NF-κB pathway results in the nuclear translocation of p52–RelB dimers after phosphorylation-dependent processing of p100 (NF-κB2). It is strictly dependent on IKKα dimers that are activated by certain cytokines including lymphotoxin-β (LTβ), CD40 ligand (CD40L), and the B-cell-activating factor BAFF/Blys. Activation of the alternative pathway turns on a gene expression program involved in the development and maintenance of secondary lymphoid organs and adaptive immunity.

NF-κB Activation

There are two major signaling pathways leading to nuclear translocation and thereby activation of NF-κB proteins. The most frequent, observed in all cell types, is the classical or canonical NF-κB activation pathway (Fig. 2). This pathway is activated by proinflammatory cytokines such as tumor necrosis factor-α (TNFα) (►tumor necrosis factor) and interleukin-1 (IL-1), as well as pathogen-associated molecular patterns (►PAMPs). These effector molecules act through different receptors belonging to the tumor necrosis factor receptor (TNF-R) and Toll-like receptor (TLR)-interleukin-1 receptor (IL-1R) superfamilies (►Toll-like receptors) and activate a large multiprotein complex, the IκB kinase (►IKK) complex. The most common form of this complex consists of two catalytically active protein kinases, IKKα (or IKK1) and IKKβ (or IKK2), forming heterodimers, which are associated with at least two molecules of the IKKγ regulatory subunit (also called NEMO for “NF-κB essential modulator”). The function of the activated IKK complex is to catalyze the rapid ►phosphorylation of the IκBs at two serine residues located in their amino-terminal regulatory domain (Fig. 1b) triggering their ubiquitination-mediated degradation by the 26S proteasome. NF-κB dimers (predominantly RelA–p50 dimers) are released, translocated to the nucleus where they bind to DNA and activate gene transcription.

A second NF-κB activation pathway, called the alternative or noncanonical pathway is activated in response to certain cytokines that belong to the TNF cytokine family including lymphotoxin-β (LTβ), CD40 ligand (CD40L), and the B-cell-activating factor BAFF (Fig. 2). This pathway strictly depends on the activation of IKKα homodimers, which phosphorylate NF-κB2 at two serine residues located at the carboxy-terminal end of the protein. Phosphorylation of these sites induces the ubiquitin-dependent processing of NF-κB2 to generate the mature p52 protein (amino-terminal portion of NF-κB2) while the carboxy-terminal part is degraded by the 26S proteasome. As NF-κB2 is mainly associated with RelB, its processing leads to the release and nuclear translocation of p52–RelB heterodimers.

NF-κB and Immunity

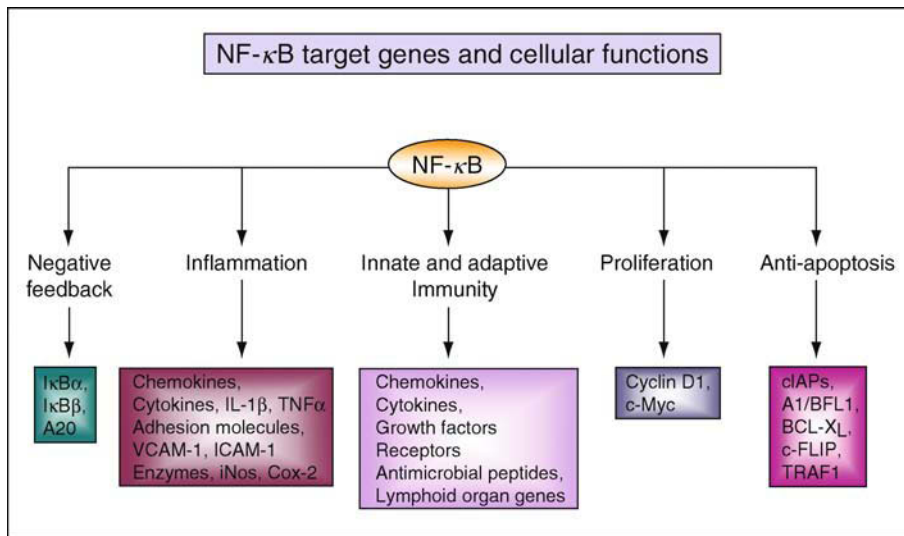
NF-κB regulates both innate and adaptive immune responses (►immune defense) [2]. Understanding the function of NF-κB in the development, maintenance, and activation of cells from the immune system (including hematopoietic cells, macrophages, dendritic cells, B and T lymphocytes) has greatly benefited from the analysis of knockout mice in which individual NF-κB family members were defective.

The innate immune response is initiated once the host detects the presence of foreign pathogens. This recognition step is performed by specialized cells of

the innate immune system, the macrophages, and the dendritic cells. These cells express at their surface or intracellularly, a number of pattern recognition receptors (►PRRs), each of which has the ability to recognize specific pathogen-derived substances called (►PAMPs) characteristics of various classes of microbes including bacteria, viruses, and parasites. Activation of the classical NF- κ B signaling pathway is essential to this acute phase of antimicrobial defense. Defective NF- κ B activity in mice and humans is often associated with susceptibility to microbial infections. Indeed, activation of NF- κ B dimers (mainly RelA-p50) induces transcription of genes encoding cytokines (►cytokines), chemokines, adhesion molecules, and antimicrobial peptides (►defensins), all of which are essential to build the innate immune response, clear the pathogen, and initiate the adaptive immune response (Figs 2 and 3).

NF- κ B is also crucial for the proper functioning of the adaptive immune system not only by acting on the immune cells themselves but also by participating in the development and organization of the secondary lymphoid organs (lymph nodes, spleen, and Peyer's patches), in which both B and T lymphocytes undergo maturation and activation. NF- κ B proteins have an important role in lymphocyte development and

homeostasis, primarily through regulation of the balance between cell proliferation and cell death (Fig. 3). The antiapoptotic function of NF- κ B is required during early development and expansion of B-cells, which occurs in the bone marrow and T-cells, which takes place in the thymus. Different genetic approaches have uncovered the involvement of NF- κ B proteins in T-cell activation and function. For instance, NF- κ B is involved in the production of interleukin-18 (IL-18) and interferon- γ (INF- γ), two cytokines required to develop the cellular or T helper 1 (T_H1) immune response. Activation of both the canonical and noncanonical NF- κ B pathways controls B-cell maturation and function such as immunoglobulin class switching and cytokine production. Recently, efforts have concentrated on understanding the contribution that individual NF- κ B family members make to the development of secondary lymphoid organs and on the identification of specific NF- κ B target genes activated in the different cell types (stromal, dendritic, and lymphoid) implicated in this developmental program. Organogenic stimuli such as LT β activate both the canonical and the non-canonical pathways although with different kinetics. This leads to sequential activation of different subsets of NF- κ B target genes. Expression of proinflammatory



Nuclear Factor Kappa B. Figure 3 NF- κ B cellular functions and target genes. NF- κ B contributes to the induction of multiple target genes that can be divided into five major functional classes, which specify diverse cellular functions. NF- κ B controls its own activity by inducing the expression of its regulators, the I κ B proteins and establishing a negative feedback regulatory loop. NF- κ B induced multiple genes whose products are mediators of inflammation such as proinflammatory cytokines (TNF α and IL-1), adhesion molecules (VCAM-1, ICAM-1, E-selectin), and the inflammatory enzymes, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). NF- κ B has critical immunoregulatory functions both in the innate immune defense against pathogens and in the development, maintenance, and activation of the adaptive immunity. The antiapoptotic function of NF- κ B requires the activation of genes whose products are implicated in the inhibition of caspase activity such as the cellular inhibitors of apoptosis (cIAPs) and the cellular FLICE-inhibitory protein (cFLIP) or are negative regulators of the mitochondrial cell death program such as the Bcl-2 family protein members, Bfl-1/A1 and Bcl-X_L. NF- κ B plays a role in cell proliferation and differentiation in particular by regulation the expression of the genes encoding the cell-cycle regulator, cyclin D1, and the oncogene cMyc.

chemokines such as eotaxin, Rantes, and the monocyte chemotactic protein-1 (MCP-1) is under the control of the classical pathway while induction of organogenic chemokines such as SLC (secondary lymphoid tissue chemokine) or SDF-1 (stromal cell-derived factor-1) requires p52-RelB dimers activated in the noncanonical pathway.

NF- κ B, Inflammation and Inflammatory Diseases

NF- κ B is one of the most important regulators of inflammation [2]. Proinflammatory stimuli such as those elicited by the cytokines TNF α and IL-1, are the most potent activators of NF- κ B and trigger a proinflammatory gene expression program consisting of transcription of genes encoding proinflammatory cytokines (TNF α , IL-1, and IL-6), chemokines, adhesion molecules, inflammatory enzymes (cyclooxygenase-2, COX-2; inducible nitric oxide synthase, iNOS), and **matrix metalloproteinases (MMPs)** (**matrix metalloproteases**) (Figs 2 and 3). Massive production of proinflammatory mediators (TNF α and IL-1) amplifies the inflammatory response despite the activation of at least two negative feedback mechanisms mediated by induced expression of the NF- κ B inhibitors (IkBs) and of the ubiquitination enzyme A20. Recruitment of inflammatory cells to the site of inflammation and tissue destruction also occurs quite rapidly after NF- κ B activation. High NF- κ B activity is observed at the center of inflammation in several diseases affecting different tissues or organs including rheumatoid arthritis (**inflammation and rheumatoid arthritis**), inflammatory bowel disease, asthma, and psoriasis. Specific inhibition of NF- κ B activity has been shown to substantially reduce the inflammatory response in different animal models of inflammatory diseases. Most antiinflammatory drugs used to treat inflammatory diseases have direct or indirect effects on NF- κ B activity.

NF- κ B and Programmed Cell Death

Programmed cell death, which includes [3] apoptosis and necrosis is largely controlled by NF- κ B (**apoptosis**). The classical pathway is responsible for inhibition of programmed cell death (PCD) triggered by activation of several receptors including type I and type II TNF-receptors (TNF-RI and TNF-RII), TLR4 and T- and B-cell receptors, which are able to generate both survival and death signals once activated. NF- κ B favors survival by inducing the expression of several antiapoptotic genes whose products act at different steps along the receptor-mediated or the mitochondrial proapoptotic cascades. These antiapoptotic proteins include the cellular FLICE-inhibitory protein (c-FLIP), the cellular inhibitor of apoptosis (c-IAP1 and c-IAP2), the X-chromosome-linked IAP (XIAP), the TNF receptor-associated factor-1 and -2 (TRAF1 and TRAF2) and several members of

the Bcl-2 family such as the Bcl-2 homologs Bfl-1/A1 and Bcl-X_L. The first clear evidence that NF- κ B is an effective inhibitor of apoptosis came from the analysis of RelA-deficient mice that die during embryonic development from massive liver apoptosis. Defective NF- κ B activation is unable to protect the hepatocytes from the toxic effects caused by the massive release of TNF α that occurs in the liver of a developing embryo. A similar phenotype is observed in mice defective in IKK β and IKK γ , the two components of the IKK complex that are crucial for activation of the antiapoptotic program mediated through the classical NF- κ B pathway. The alternative pathway also supports an antiapoptotic program important for survival of premature B-cells and development of secondary lymphoid organs as described above. This protective effect of NF- κ B against apoptosis has a dramatic side effect in cancer cells as it antagonizes the killing potential of most anticancer drugs that also activate NF- κ B. In addition, constitutive NF- κ B activity is observed in various solid and hematologic tumors. NF- κ B also influences the tumor microenvironment by producing proinflammatory cytokines that promote tumor cell growth and therefore appears as a major factor in the development of inflammation-associated cancers (**cancer**). The development of specific NF- κ B inhibitors represents an attractive therapeutic approach in cancer therapy.

Drugs

There are several hundred reported NF- κ B inhibitors (see www.nf-kb.org for a complete and updated list). These inhibitors include natural products, chemicals, metabolites, and synthetic compounds. A large majority of these products, in particular commonly used anti-inflammatory drugs such as corticosteroids and the nonsteroidal antiinflammatory drugs (NSAIDs) aspirin, sulindac, ibuprofen and sulphasalazine, have the ability to partially inhibit NF- κ B activity in cell culture. However, the precise mechanism of action and the specific molecular targets of most of these inhibitors remain unclear.

Several strategies targeting different steps of the signaling cascade can be used to inhibit NF- κ B activation and function [4,5]. One general approach is to interfere with NF- κ B nuclear activity by altering dimerization, nuclear translocation, binding to DNA, or interaction with the transcriptional machinery. This includes the use of decoy oligonucleotides that have κ B sites and compete NF- κ B dimers out of their target promoters or cell-permeable peptides able to block specifically the nuclear translocation of NF- κ B.

A second strategy that is more likely to succeed is to interfere with the process of activation of NF- κ B by blocking the activation of the IKK complex or the degradation of IkBs. Pharmaceutical companies are investing considerable effort in the development of

proteasome inhibitors, many of which inhibit I κ B degradation and thereby NF- κ B nuclear translocation. However, as the proteasome is involved in the degradation of many different polyubiquitinated proteins, finding an inhibitor specific to NF- κ B activation is extremely difficult. One proteasome inhibitor, bortezomib has entered clinical trial for the treatment of solid and hematologic tumors. It is still unclear whether its therapeutic effect in cancer treatment is entirely or partially due to inhibition of NF- κ B.

A more promising approach to selectively inhibit NF- κ B activation seems to rely on the development of small-molecule inhibitors targeting the activity of signaling kinases in particular IKK α and IKK β . The pharmaceutical industry is actively screening for selective inhibitors of the catalytic activity of IKK α and IKK β . The development of IKK α -specific inhibitors has been hampered in part by an incomplete understanding of its specific function in NF- κ B activation and the lack of specific cell-based assays to test the potential inhibitors. By contrast, the development of specific IKK β inhibitors (see www.nf-kb.org) is progressing rapidly and numerous compounds are in the preclinical phase and their effects and toxicity are being evaluated in various in vitro assays and animal models of inflammatory diseases and cancer.

- Immune Defense
- Inflammation
- Apoptosis

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Nuclear Factor of Activated T Cells Cytosolic NFATc

- NFAT Family of Transcription Factors

Nuclear Localization Signal

Sequence of amino acids that determine the transport of proteins into the nucleus. Although there is no clear consensus, nuclear localization signals tend to be rich in positively charged residues, which allow interaction with proteins from the nuclear import machinery (i.e., importins).

Nuclear Pore Complex

The nuclear pore complex, located in the nuclear envelope, contains more than 50 proteins. It allows diffusion of small proteins between cytoplasm and nucleoplasm. Larger molecules (>50kD) are selectively transported by an energy-dependent mechanism.

- Bacterial Toxins
- Small GTPases

Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes

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Definition

Cytochrome P450 (CYP) induction is the process whereby cellular or tissue levels of one or more ►cytochrome P450 enzymes are increased via de novo protein synthesis in response to treatment with certain drugs (e.g., phenobarbital) and environmental chemicals, which are designated P450 inducers. This leads to an increase in the capacity for P450-catalyzed oxidative metabolism of both xenochemicals (i.e., drugs and other foreign chemicals) and endogenous lipophilic substrates. Other drug-metabolizing enzymes, such as drug conjugation enzymes, e.g., glutathione S-transferases and UDP-glucuronyltransferases, are also subject to induction by classic P450 inducers.

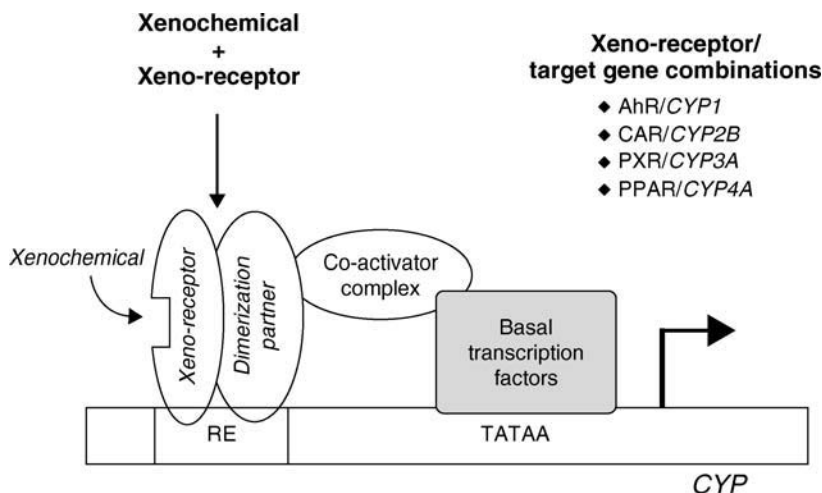
Basic Characteristics

P450 induction can occur in many cell types and tissues, but is most prominent in liver, a major organ for metabolism of steroids, drugs, and environmental chemicals. Many of the inducible P450s are active catalysts of drug metabolism, and P450 induction typically enhances the capacity for foreign chemical biotransformation, resulting in a shorter half-life and more rapid clearance of the compound from the body. Consequently, P450 induction can have a major impact on many processes, including P450-dependent drug metabolism, pharmacokinetics, - and drug-drug interactions; the toxicity and carcinogenicity of foreign chemicals; and the activity and disposition of endogenous steroids and certain other hormones. Although some P450 substrates also serve as P450 inducers, there is no general relationship between the ability of a chemical to induce a particular P450 enzyme and its ability to serve as a substrate of the same P450.

Of the 57 known human P450s, at least ten are subject to induction by xenochemicals. In most cases, the induction of P450 protein and enzyme activity occurs by a mechanism that involves increased transcription of the corresponding P450 gene. Members of four cytochrome P450 gene families, *CYP* families 1, 2, 3 and 4, are induced by receptor-dependent transcriptional mechanisms (Fig. 1). P450 genes belonging to the *CYP1* gene family and the *CYP2S1* gene are induced by the aryl hydrocarbon (Ah) receptor (also known as dioxin receptor), which is a transcription factor and a member of the basic Helix-Loop-Helix/Per-Arnt-Sim (bHLH/PAS) family [1]. In contrast, the induction of

select members of the *CYP2*, *CYP3*, and *CYP4* gene families is mediated by nuclear receptor superfamily members known as constitutive androstane receptor (CAR; induction of select *CYP2* genes) [2, 3], pregnane X receptor (PXR; induction of *CYP3A* genes) [3], and peroxisome proliferator-activated receptor α (PPAR α ; induction of *CYP4A* genes) [4].

CAR is an **orphan nuclear receptor** that mediates the widely studied induction of *CYP2B* genes by phenobarbital and many other “phenobarbital-like” lipophilic drugs and other chemicals [5]. PXR activates *CYP3A* genes in response to diverse chemicals, including certain drugs, natural products, and natural and synthetic steroids [5]. PPAR α mediates the induction of fatty acid hydroxylases of the *CYP4A* family by many acidic chemicals classified as nongenotoxic carcinogens and peroxisome proliferators (4). These three xenochemical receptors are most abundant in liver, where they may be activated by endogenous ligands. CAR and PXR are also expressed in the intestine, where they mediate P450 induction as well. The discovery of endogenous ligands for CAR (androstanes, which decrease basal receptor activity and serve as inverse agonists), PXR (certain pregnenolone derivatives, bile acids, and other steroids), and PPAR α (specific prostaglandins and other fatty acid metabolites) suggests that these three nuclear receptors play an important role in modulating liver gene expression in response to endogenous metabolic or hormonal stimuli, in addition to their more obvious role in modulating liver drug and xenochemical metabolism by induction of cytochromes P450 and other enzymes of foreign compound metabolism.



Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes. Figure 1 General mechanism for transcriptional activation of *CYP* genes by xenochemicals that activate their cognate xeno-receptor proteins. In the case of Ah receptor, the receptor's heterodimerization partner is Arnt, whereas in the case of the nuclear receptors CAR, PXR, and PPAR α , the heterodimerization partner is RXR. The coactivator and basal transcription factor complexes shown are each comprised of a large number of protein components.

CYP1 Induction via Ah Receptor

The Ah receptor is localized in the cytosol in the basal state, where it exists in a complex containing heat shock protein 90 (Hsp90), a chaperone protein, and co-chaperone proteins (e.g., the hepatitis B virus X-associated protein [XAP2] and p23). Ah receptor is activated upon binding a ligand in the cytosol. The ligand-activated receptor then translocates to the nucleus, where it dissociates from the chaperone and co-chaperone proteins and heterodimerizes with a nuclear protein, Ah receptor nuclear translocator (ARNT). The Ah receptor-ARNT heterodimer binds to DNA enhancer sequences ("dioxin-response elements" or DREs) found upstream of *CYP1* and other Ah receptor-inducible genes involved in biotransformation, cell proliferation, and differentiation, and stimulates target gene transcription. The overall pathway for receptor activation is conserved in many cell types and across species and accounts for the induction of *CYP1* genes by a large number of aromatic hydrocarbons, including important environmental carcinogens found in auto emissions and cigarette smoke. Halogenated and polycyclic aromatic hydrocarbons are prototypic Ah receptor ligands, but certain planar compounds, including dietary plant constituents, can also bind to the Ah receptor, although with varying affinities. The details of the molecular mechanisms of Ah receptor signaling are not fully understood, but they appear to involve multiple events, including (i) interaction of the Ah receptor with various coactivators and corepressors, NF- κ B, and the retinoblastoma protein; (ii) activation of various protein kinases; and (iii) phosphorylation of the Ah receptor.

Role of CAR in CYP2B Induction and other Phenobarbital Responses

The orphan nuclear receptor CAR is the key-regulated transcription factor that mediates the effects of phenobarbital and phenobarbital-like inducers on liver *CYP2B* and other genes [2, 3]. CAR is localized in the cytosol in the basal state (i.e., in the absence of a ligand or an activator), where it is phosphorylated on serine 202 and is in a complex with Hsp90 and a co-chaperone protein known as cytoplasmic CAR retention protein (CCRP). CAR is translocated from the cytosol to the nucleus in response to stimulation of cells by phenobarbital and other CAR activators. This process involves the recruitment of protein phosphatase 2A (PP2A), perhaps in response to activation of AMP-activating protein kinase by phenobarbital. The dephosphorylation of CAR by PP2A allows CAR to dissociate from the CAR-Hsp90-CCRP complex and translocate into the nucleus by a process that is not well understood. CAR binds to specific DNA response elements (PBREs), found upstream of *CYP2B* and other phenobarbital-inducible genes, as a heterodimer with the nuclear receptor ►retinoid X receptor (RXR), which serves as a common

heterodimerization partner for many orphan nuclear receptors, including PXR and PPAR α . The CAR-RXR complex then recruits coactivator complexes (Fig. 1), leading to an increase in the rate of transcription of CAR target genes.

CAR nuclear translocation and (CAR-RXR)-PBRE binding are strongly enhanced in liver in vivo following administration of phenobarbital. However, unlike classical nuclear receptor agonists, phenobarbital does not bind to CAR's COOH-terminal ligand-binding domain (LBD). Mouse *CAR* gene knockout studies demonstrate that CAR is essential, not only for induction of the highly inducible *CYP2B* genes, but also for the multiple pleiotropic responses associated with exposure to phenobarbital and phenobarbital-like inducing agents. These include the induction of many genes involved in xenobiotic transport and biotransformation and repression of the expression of certain genes involved in energy metabolism. CAR is also required for various pathophysiologic effects of phenobarbital in the liver (e.g., hepatomegaly, enhanced hepatocyte proliferation) and for toxicologic or carcinogenic responses that are characteristic of phenobarbital-treated liver (e.g., acetaminophen- and cocaine-induced hepatotoxicity and liver tumor promotion).

CYP3A Induction by PXR

PXR is the major transcription factor that mediates the induction of CYP3A enzymes, most notably CYP3A4, the most abundant P450 enzyme in human liver. CYP3A4 is highly expressed in liver and intestine, in which it metabolizes structurally diverse drugs, environmental chemicals, endogenous steroid hormones, and lipophilic bile acids. The high level of expression of CYP3A4, coupled with its broad substrate specificity, and widespread inducibility following exposure to diverse steroids, antibiotics, and other pharmacological agents that activate PXR, gives rise to many CYP3A-based drug interactions.

PXR was initially thought to reside exclusively in the nucleus, but subsequent studies identified PXR in the cytosol. In contrast to CAR, PXR has little or no intrinsic basal transcriptional activity in the absence of ligand. Similar to CAR, the cytosolic form of PXR exists in a complex with Hsp90 and CCRP. Induction of CYP3A and other PXR target genes requires nuclear translocation of PXR, but the details of how this occurs remain to be elucidated. Activated PXR, which is in a heterodimeric complex with RXR α and binds various coactivators, binds to specific DNA response elements adjacent to PXR target genes, enabling it to stimulate gene transcription.

Major species differences characterize the induction of CYP3A enzymes by drugs, steroids, and other chemicals. These species differences are a direct result

of the species-dependent activation of PXR by individual PXR ligands (Fig. 2). Human PXR but not mouse PXR is activated by rifampicin and other xenochemicals that preferentially induce *CYP3A* genes in human cells and tissues, whereas mouse PXR but not human PXR is activated by the synthetic steroid pregnenolone 16 α -carbonitrile (PCN). Mouse PXR gene knockout studies establish PXR as the major mediator of CYP3A induction by various xenochemicals. Moreover, a human pattern of *CYP3A* inducibility can be achieved when the endogenous mouse PXR gene is replaced by its human PXR counterpart. Mouse and human PXRs exhibit an uncharacteristically high (~25%) amino acid divergence within the LBD, suggesting that these rodent and human PXRs are unusually divergent orthologs whose evolution reflects their adaptation to the unique dietary constituents and distinct endogenous steroid profiles of each species.

PXR may serve as a broadly based “steroid and xenobiotic sensor” whose intrinsic physiologic function is to stimulate synthesis of CYP3A enzymes that catabolize endogenous steroidal substrates. This possibility is supported by the striking responsiveness of PXR to endogenous steroids belonging to several distinct classes (pregnanes, estrogens and corticoids) and by the catalysis by many CYP3A enzymes of 6 β -hydroxylation reactions using diverse steroidal substrates, including androgens, corticoids, progestins, and bile acids. PXR plays a key role in bile acid homeostasis, as shown by the decreased production and increased hepatic uptake, and detoxification of cholestatic bile acids, such as lithocholic acid, which is mediated by PXR. The activation of PXR by bile acids

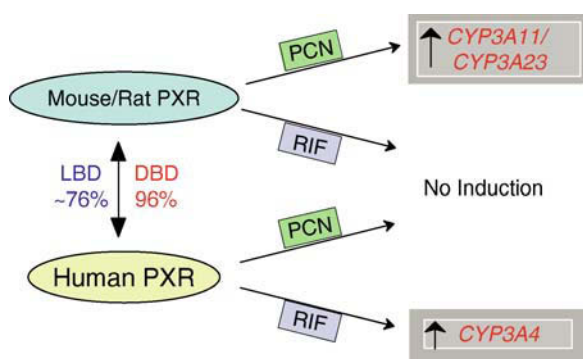
in liver leads to (i) decreased expression of *CYP7*, cholesterol 7 α -hydroxylase, which catalyzes a key rate-limiting reaction of bile acid biosynthesis; (ii) increased expression of the transporter *Oatp2*, which increases hepatic uptake of bile acids from the sinusoidal blood; and (iii) induction of CYP3A enzymes that detoxify lithocholic acid by catalyzing its 6-hydroxylation.

PPAR: Xenochemical Induction of CYP4A Enzymes and Role in Rodent Hepatocarcinogenesis

CYP4A enzymes catalyze the oxygenation of biologically important fatty acids, including arachidonic acid and other eicosanoids. *CYP4A* gene transcription can be activated in both liver and kidney by a range of acidic drugs and other xenochemicals, including hypolipidemic fibrate drugs, phthalate ester plasticizers used in the medical and chemical industries, and other environmental chemicals. These CYP4A inducers are classified as peroxisome proliferator chemicals because they markedly induce liver peroxisomal enzymes, leading to a dramatic increase in both the size and the number of liver cell peroxisomes.

PPAR α is the nuclear receptor responsible for *CYP4A* induction, peroxisomal enzyme induction, and hepatic peroxisome proliferation [4]. The tissue distribution of PPAR α (liver > kidney > heart > other tissues) mirrors the responsiveness of these tissues to peroxisome proliferator chemicals. *CYP4A* induction in liver and kidney and hepatic peroxisome proliferation are both abolished in PPAR α gene knockout mice, demonstrating the essential role of PPAR α for these responses in vivo. The general mechanism of PPAR α activation is similar to that of other nuclear receptors. PPAR α is found in the nucleus in the basal state as a complex with corepressor proteins. Ligand binding leads to dissociation of PPAR α from its corepressor proteins, heterodimerization with RXR α , recruitment of coactivators, and binding to functional DNA response elements, referred to as peroxisome proliferators response elements (PPREs), in the 5'-flank of *CYP4A* and other target genes, resulting in stimulation of gene transcription. PPAR α -RXR complexes bound to PPREs can be synergistically activated by the combination of a PPAR α ligand with the RXR ligand 9-*cis*-retinoic acid.

Persistent activation of PPAR α can induce the development of hepatocellular carcinoma in susceptible rodent species by a nongenotoxic mechanism, i.e., one that does not involve direct DNA damage by peroxisome proliferator chemicals or their metabolites. This hepatocarcinogenic response is abolished in mice deficient in PPAR α , underscoring the central role of PPAR α , as opposed to that of two other mammalian PPAR forms (PPAR γ and PPAR δ), in peroxisome proliferator chemical-induced hepatocarcinogenesis. Other toxic responses, such as kidney and testicular toxicities caused by exposure to certain phthalate



Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes. Figure 2 Species-specificity of PXR's CYP3A induction response. Shown are the amino acid sequence identities of the COOH terminal ligand-binding domain (LBD) and the central DNA-binding domain (DBD) of rodent and human PXR. *CYP3A11* and *CYP3A23* are mouse and rat P450 3A genes, respectively, whereas *CYP3A4* is a human P450 3A gene. PCN, pregnenolone 16 α -carbonitrile; RIF, rifampicin.

di-ester plasticizers, are not abolished in PPAR α -deficient mice, raising the possibility that the latter toxicities may be mediated by PPAR γ or PPAR δ .

Pharmacological Relevance

Nuclear Receptors in Drug Metabolism and Drug Development

The identification of specific nuclear receptors as molecular targets of P450 inducers impacts drug metabolism and drug development in several important ways:

1. Drug interactions, often associated with interindividual differences in drug metabolism, are a major contributor to idiosyncratic drug responses, which can sometimes be fatal. P450 induction, especially the induction of CYP3A enzymes via PXR, can contribute significantly to interpatient differences in drug metabolism. High throughput screens for P450 inducers that activate Ah receptor, CAR, PXR, and PPAR α have been developed and can readily be applied to characterize the P450 induction potential of drugs currently used in the clinic, as well as investigational drugs and lead compounds under development. These efforts may help to predict, and thereby avoid, drug interactions associated with P450 induction.
2. Interindividual differences in the function and expression of nuclear receptors and their accessory proteins, reflecting either genetic or epigenetic factors, may represent another set of determinants of interindividual differences in pharmacokinetics and possibly pharmacodynamics. Further elucidation of the factors that regulate cellular nuclear receptor levels (e.g., glucocorticoids, which increase expression of PXR in human hepatocytes) and the identification of genetic polymorphisms that impact receptor expression, ligand-binding specificity or transcriptional activity are also likely to be important.
3. Receptor proteins involved in the induction of cytochromes P450 and other enzymes of drug metabolism may serve as novel drug targets. Examples of established nuclear receptor drug targets include PPAR α , which is a target of hypolipidemic fibrate drugs, and PPAR γ , which is targeted by anti-type II diabetes drugs of the thiazolidinedione class. CAR and PXR are also therapeutic targets, based on the role of CAR activators in the treatment of jaundice and PXR activators in the relief of cholestasis, associated with hepatotoxic bile acids. PXR antagonists might be used to block CYP3A autoinduction responses, which can substantially shorten the plasma half-life of a drug that simultaneously serves as a CYP3A inducer and a CYP3A substrate, which is a characteristic of several AIDS protease inhibitors and the anticancer drug ifosfamide. The finding that genes encoding liver and intestinal drug transporters are also

targets of CAR and PXR presents not only additional opportunities, but also additional challenges.

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Nuclear Receptors

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Synonyms

Transcription factors

Definition

Nuclear receptors (NRs) are members of a large superfamily of evolutionarily related DNA-binding transcription factors that regulate programs involved in a broad spectrum of physiological phenomena.

The sequencing of the human genome has led to the identification of 48 NRs. Sequence alignment and phylogenetic tree construction resulted in a classification of the human NR family by sequence, but not necessarily by pharmacology, into six evolutionary groups of unequal size. Trivial names have been proposed for some time. A proposition for a systematic numbering system and receptor code, supporting the trivial names,

was made in conjunction with NC-IUPHAR, and has been accepted (Table 1).

Among these NRs, ligands have been identified for only 24 family members. These receptors are ligand-dependent transcriptional factors that respond directly to a large variety of hormonal and metabolic substances that are hydrophobic, lipid soluble, and of small size. Some of these ►**ligands**, as retinoic acid or estradiol, exert their pleiotropic effects through different receptor ►**subtypes** that originate from different genes. Moreover, for each subtype several ►**isoforms** can exist. The other class of NRs is the so-called orphan receptors, for which regulatory ligands are still unknown or may not exist (true orphans), or for which candidates have only recently been identified (adopted orphans).

Basic Mechanisms

Despite a highly evolutionarily conserved structural organization, the function and the mode of action of NRs are very diverse and each receptor has crucial and nonredundant roles, notably in the regulation of many biologically important processes in growth, development, and homeostasis. NRs modulate transcription through several distinct mechanisms, which include both activation and repression activities. These activities, making NR signaling remarkably complex, can be genomic or nongenomic, be ligand dependent or independent, and mediate gene repression, the release of gene repression, gene activation, or gene transrepression by recruiting transcriptional machineries and epigenetic enzymes that modulate the chromatin at target sites (Fig. 1). NRs can also be target of other signaling pathways that modify the receptor post-translationally and affect its function. While some NRs are translocated to the nucleus after synthesis, regardless of the presence of ligand, others such as androgen receptor (AR, NR3C4), mineralocorticoid receptor (MR, NR3C2), progesterone receptor (PR, NR3C3), or glucocorticoid receptor (GR, NR3C1) are located predominantly in the cytoplasm in the absence of ligand. Binding of an ►**agonist** or some ►**antagonists** to these cytosolic receptors induces a nuclear translocation.

Modular Organization

All NR proteins exhibit a characteristic modular structure that consists of five to six domains of homology (designated A–F, from the N-terminal to the C-terminal) based on regions of conserved sequence and function (Fig. 2).

The N-terminal A/B region whose structure has not yet been defined contains a transcriptional activation function, referred to as activation function 1 (AF-1), which can operate autonomously. The length and sequence of the A/B region in the different NRs are highly variable, revealing a very weak evolutionary

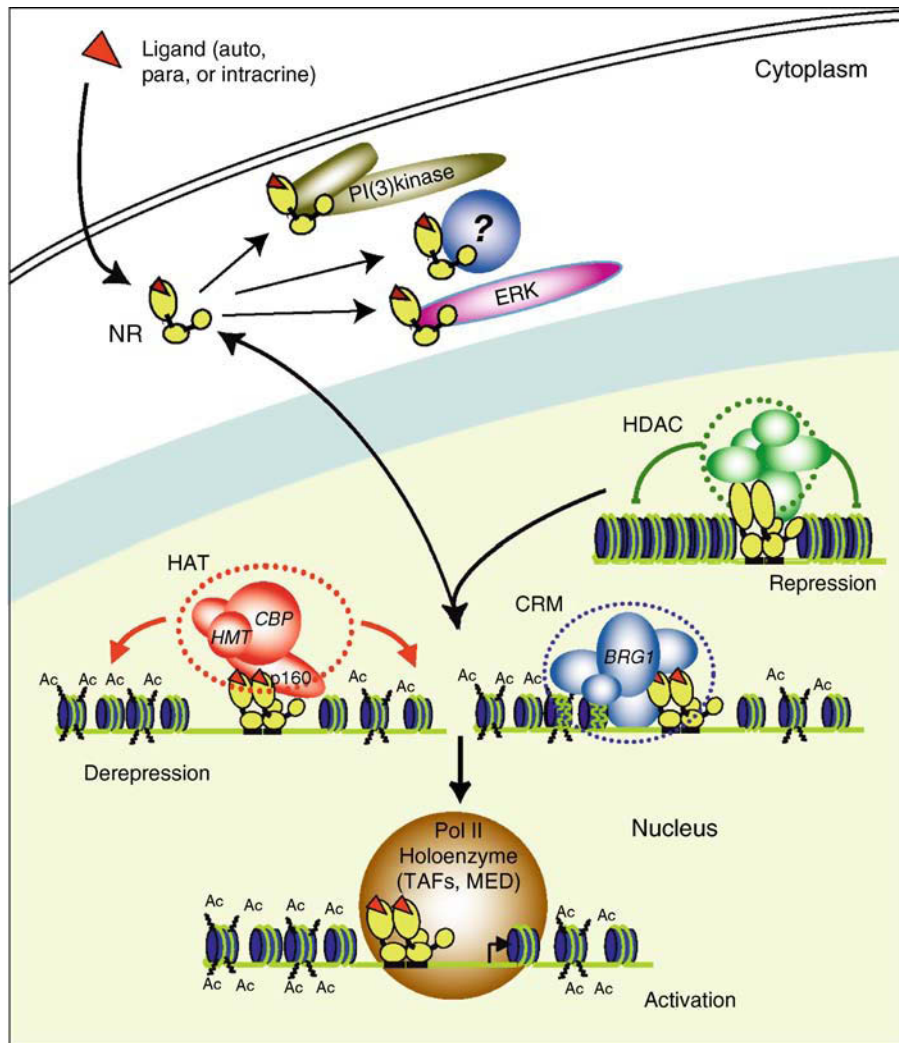
conservation. This domain is the target of posttranslational modifications and can interact with cofactors such as coactivators or other transcription factors.

The central C region of the NRs harbors the DNA-binding domain (DBD) and corresponds to the most conserved domain. Through this domain, NRs bind to specific DNA sequences called hormone response elements (HREs) (except for DAX1 (NR0B1) and SHP (NR0B2), which do not harbor a DBD). The DBD consists of a highly conserved 66-residue core made up of two typical cysteine-rich zinc-finger motifs, two α -helices, and a COOH extension. It includes several sequence elements, referred to as P-, D-, T- and A-boxes, that define or contribute to the response element specificity. This specificity is generated by dimerization interfaces within the DBDs and direct contacts with the DNA including residues flanking the DNA core recognition sequence. The D region, which is a poorly conserved domain, is considered to serve as a hinge between the DBD and the ligand-binding domain (LBD), allowing flexibility of the DBD–LBD linkage. Therefore, it might permit the DBDs and the LBDs to adopt different conformations without creating steric hindrance problems. This domain also harbors a nuclear localization signal (NLS), or at least some elements of a functional NLS.

The E region, the LBD, which is less conserved than the DBD, is functionally complex, as it mediates ligand binding, dimerization, and contains a ligand-dependent ►**transactivation** function. The LBD contains four structurally distinct but functionally linked surfaces: (i) a dimerization surface, which mediates interaction with partner LBDs, (ii) the ligand-binding pocket (LBP), which interacts with diverse lipophilic small molecules in the case of liganded NRs, (iii) a ►**coregulator**-binding surface that binds to regulatory protein complexes, which modulate positively or negatively transcriptional activity, and (iv) an activation function helix, termed AF-2, which mediates ligand-dependent transactivation. Within the AF-2, the integrity of a conserved amphipathic α -helix called AF-2 activation domain (AD) has been shown to be required for ligand-dependent transactivation and ►**coactivator** recruitment. Moreover, some NRs can also interact with transcriptional ►**corepressors** through their LBD. Some members of the NR superfamily, the steroid receptors, interact also with heat-shock proteins through their LBD. The LBD crystal structures of all classic liganded receptors and adopted orphan receptors have been determined. These crystal structures revealed a common fold comprising 12 α -helices (H) and a short β -turn (s1–s2), arranged in three layers to form an antiparallel “ α -helical sandwich.” The lower part of the LBD harbors a variable region, which contains the LBP which is an important structural feature of NRs, at least for the liganded receptors, since the first step of receptor activation is initiated by ligand binding. LBP is lined with mostly hydrophobic amino acids and few polar residues

Nuclear Receptors. Table 1 Human nuclear receptors

Names	Nomenclature	Ligand
TR α	NR1A1	Thyroid hormones
TR β	NR1A2	Thyroid hormones
RAR α	NR1B1	Retinoic acid
RAR β	NR1B2	Retinoic acid
RAR γ	NR1B3	Retinoic acid
PPAR α	NR1C1	Fatty acids, leukotriene B ₄ , fibrates
PPAR β	NR1C2	Fatty acids
PPAR γ	NR1C3	Fatty acids, prostaglandin J ₂ , thiazolidinediones
Rev-erba	NR1D1	Orphan
Rev-erb β	NR1D2	Orphan
ROR α	NR1F1	Cholesterol, cholesteryl sulfate
ROR β	NR1F2	Retinoic acid
ROR γ	NR1F3	Orphan
LXR α	NR1H3	Oxysterols, T0901317, GW3965
LXR β	NR1H2	Oxysterols, T0901317, GW3965
FXR α	NR1H4	Bile acids, fexaramine
FXR β	NR1H5	Lanosterol
VDR	NR1I1	Vitamin D 1,25 dihydroxyvitamin D ₃
PXR	NR1I2	Xenobiotics, PCN (16 α -cyanopregnenolone)
CAR	NR1I3	Xenobiotics, phenobarbital
HNF4 α	NR2A1	Orphan
HNF4 γ	NR2A2	Orphan
RXR α	NR2B1	Retinoic acid
RXR β	NR2B2	Retinoic acid
RXR γ	NR2B3	Retinoic acid
TR2	NR2C1	Orphan
TR4	NR2C2	Orphan
TLL	NR2E2	Orphan
PNR	NR2E3	Orphan
COUP-TFI	NR2F1	Orphan
COUP-TFII	NR2F2	Orphan
EAR2	NR2F6	Orphan
ER α	NR3A1	Estradiol-17 β , tamoxifen, raloxifene
ER β	NR3A2	Estradiol-17 β , various synthetic compounds.
ERR α	NR3B1	Orphan
ERR β	NR3B2	DES, 4-OH tamoxifen
ERR γ	NR3B3	DES, 4-OH tamoxifen
GR	NR3C1	Cortisol, dexamethasone, RU486
MR	NR3C2	Aldosterone, spiro lactone
PR	NR3C3	Progesterone, medroxyprogesterone acetate, RU486
AR	NR3C4	Testosterone, flutamide
NGFIB	NR4A1	Orphan
NURR1	NR4A2	Orphan
NOR1	NR4A3	Orphan
SF1	NR5A1	Orphan
LRH1	NR5A2	Orphan
GCNF	NR6A1	Orphan
DAX1	NR0B1	Orphan
SHP	NR0B2	Orphan



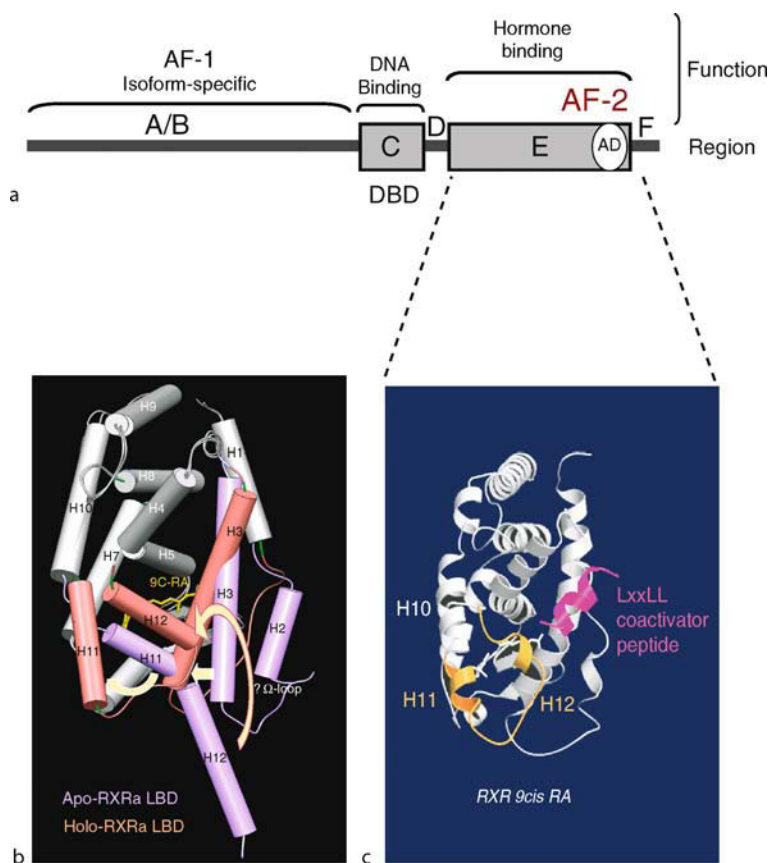
Nuclear Receptors. Figure 1 Mode of action of nuclear receptors. After diffusion through the cytoplasmic membrane, the ligand can interact with its cognate receptor where it can exert a “nongenomic effect” by interacting directly, for example, with kinases (1). The ratio between cytoplasmic and nuclear locations may vary between different receptors and is affected by the nature of a ligand. Ligand binding modulates the interaction of the receptor with a plethora of factors. In the absence of a ligand, several nuclear receptors are believed to be bound to the regulatory regions of target genes as corepressors or HDAC (histone deacetylase) complexes (2). Histone deacetylation is responsible for the chromatin condensation which accounts for the gene silencing effect of unliganded receptors. Ligand binding releases the HDAC complex (3) and results in the recruitment of histone acetyltransferase (HAT) and chromatin-remodeling (CRM) complexes (4). The temporal order and requirement of these complexes may occur in a receptor, target gene in a cell-specific fashion. As the last step (5), the polymerase II holoenzyme, comprising the TAF and MEDIATOR complexes in addition to the enzyme, is recruited and it increases the frequency of transcription initiation.

at the deep end of the pocket near the β -turn act as anchoring points for the cognate ligand or play an essential role in the correct positioning and enforce the selectivity of the pocket. The specificity of ligand binding is also determined by the shape of the LBP, which can vary greatly from receptor subtype to subtype.

The C-terminal F region, that is contiguous with the E domain, is not present in all receptors, and its function is poorly understood.

DNA Recognition

An essential step of NR action is the interaction of these receptors with the specific DNA sequence HREs. Indeed, HREs position the receptors, and the transcriptional complexes recruited by them, close to the target genes. HREs are bipartite elements that are composed of two hexameric core half-site motifs. These consensus nucleotide sequences form direct, indirect, or inverted repeats, which consist of two half-sites separated



Nuclear Receptors. Figure 2 Schematic illustration of the structural and functional organization of nuclear receptors. (a) The evolutionarily conserved regions C and E are indicated as boxes (green), a black bar represents the divergent regions A/B, D, and F. Domain functions are depicted above the scheme. AF-1 Activation Function-1, which is located in the N-term A/B region, AF-2 Activation Function-2. (b) Schematic drawing of the superposition of the canonical unliganded and liganded 3D structures of nuclear receptor ligand-binding domains (LBDs). Upon ligand binding, a series of intramolecular interactions cause the repositioning of H11 in the continuity of H10, and the concomitant swinging of H12. (c) The proper repositioning of structural elements (H3, H4, L3–4, and H12) upon ligand binding allows interaction through the highly conserved α -helical LxxLL motif (in which L corresponds to Leucine and x to any amino acid) with the NR box of nuclear receptor coactivators, such as TIF2/GRIP1, SRC1, or RAC3/AIB1/ACTR.

by a short spacer. In the genome, they are arranged as a multiplicity of response elements characterized by (i) the actual nucleotide sequence of the two core motif half-sites, (ii) the number of base pairs separating them, and (iii) the relative orientation of the motifs.

The NR superfamily can be divided into subgroups on the basis of their pattern of dimerization. One group consists of the steroid receptors, all of which appear to function as homodimers that bind to a degenerate set of response elements containing inverted repeats of a hexameric half-site separated by three base pairs of spacing (IR3). Nearly all known nonsteroid receptors recognize one or two copies of the consensus DNA sequence 5'-AGGTCA-3'. Among these receptors, a major group consists of receptors that form heterodimers with the retinoid X receptors (RXRs). The various RXR heterodimers can bind to direct repeats

(DRs) with one to five base pairs of spacing, referred to as DR1–DR5. Some NRs can also bind DNA efficiently as monomers such as NGFI-B (NR4A1), Rev-erb, RORs, and SF-1 (NR5A1).

The Liganded Receptors

In the case of liganded NRs, ligand binding is the first and crucial molecular event that switches the function of these transcription factors from inactive to active state by inducing a conformational change in the LBD of the receptor (Fig. 1). This specific conformation allows the second step of NR activation that corresponds to the recruitment of coregulatory complexes, which contain chromatin-modifying enzymes required for transcription. The transcriptional coactivators are very diverse and have expanded to more than hundred in number. These include the p160 family of proteins,

CREB-binding protein (CBP) and p300, coactivator-associated arginine methyltransferase-1 (CARM1), and histone acetyl transferases (HATs). The ultimate action of liganded NRs on target genes is to enhance the recruitment and/or function of the general transcription machinery (RNA polymerase II and general transcription factors). From a structural point of view, agonists are ligands that lock the receptor in the active conformation. In contrast, antagonists should be viewed as molecules that prevent NRs to adopt this conformation. Helix 12 is a crucial component of the NR LBDs, because its ligand-induced repositioning in the agonist-bound NR contributes in a critical manner to the surfaces recognized by coactivators and thereby generates a transcriptional active AF-2 domain. Hence, the interactions between AF-2 helix 12 or residues in its proximity and the ligand is critical for the control of agonist-antagonist properties of NRs.

Some NRs exhibit a dual functionality, being able to act as silencers of transcription in the absence of ligands, due to their ability to recruit corepressor complexes at the promoters of target genes, in addition to activating transcription in the presence of agonists. This silencing function has been well established for unliganded retinoic acid and thyroid hormone receptors. Hence, nuclear receptor corepressor 1 (NCoR1) or silencing mediator for retinoid and thyroid hormone receptor (SMRT) are able to bind to these receptors and to recruit transcriptional complexes that contain specific histone deacetylases (HDACs). The ligand-induced conformational change causes dissociation of the corepressors, allowing the receptor to interact with coactivators.

The transcriptional activity of NRs is also modulated by various posttranslational modifications of the receptors themselves or of their coregulatory proteins. Phosphorylation, as well as several other types of modification, such as acetylation, SUMOylation, ubiquitinylation, and methylation, has been reported to modulate the functions of NRs, potentially constituting an important cellular integration mechanism. In addition to the modifications of the receptors themselves, such modifications have been reported for their coactivators and corepressors. Therefore, these different modes of regulation reveal an unexpected complexity of the dynamics of NR-mediated transcription.

Evidence has accumulated over the past few years that NR action is not restricted to the positive or negative regulation of the expression of cognate target genes. Indeed, these receptors together with their mediators are targets of other major signaling cascades, and reciprocally, they can affect the activity of these pathways. Hence, in response to ligands, some NRs, such as GR, regulate gene programs not only by directly binding to HREs but also through signal transduction

crosstalk, for example, by interfering with AP-1 and NF κ B activities that are the prototypes of a negative regulation. Despite the proposal of several distinct mechanisms, the molecular basis of these interferences has remained elusive and requires an unknown state of the receptor.

Another type of NR crosstalk, which has only recently been recognized, is the so-called “nongenomic” actions of several receptors that induce very rapid cellular effects. Effectively, evidence has accumulated over several decades that steroid receptors may have a role that does not require their transcriptional activation, such as modifying the activity of enzymes and ion channels. While the effects of steroids that are mediated by the modulation of gene expression do occur with a time lag of hours, steroids can induce an increase in several second messengers such as inositol triphosphate, cAMP, Ca²⁺, and the activation of MAPK and PI3 kinase within seconds or minutes. Many mechanistic details of these nongenomic phenomena remain poorly understood. Notably, controversy still exists as to the identity of the receptors that initiate the non-genomic steroid actions. However, it now appears that at least some of the reported effects can be attributed to the same steroid receptors that are known as NRs.

The Orphan Receptors

A significant number of the 48 human NRs are still considered as orphan receptors, as no physiologically relevant ligand has been found and the issue of cognate ligands for these proteins remains very controversial and unclear. Whereas a clear structural paradigm has developed to explain the activation of ligand-regulated receptors, no uniform mechanism has been proposed to account for the modulation of orphan NR activity. Nevertheless, the existence of these proteins, that raise questions about the role of ligand-binding, suggests that additional unexplored NR-mediated signaling pathways remain to be characterized.

Several recent structural studies indicate that “true” orphan receptors, that is, NRs that are not recognized by cognate endogenous ligands and in which the AF-2 helix is predisposed in the active conformation, might indeed exist. For instance, the elucidation of the 3D crystal structure of the NURR1 LBD revealed that although this LBD is folded much the same way as the other NRs, it lacks a cavity for ligand binding. Indeed, the LBP that is normally occupied by ligands in other classic NRs is entirely filled by hydrophobic amino acid side chains in NURR1 (NR4A2). Nevertheless, NURR1 can act as a transcription factor since target genes of this receptor have been identified. Therefore, NURR1, as well as NGFI-B, is not regulated by cognate ligands and might be regulated by alternative mechanisms.

Pharmaceutical Relevance

Because of the essential role played by NRs in virtually all aspects of mammalian development, metabolism and physiology, dysfunction of signaling controlled by these receptors is associated with reproductive, proliferative, and metabolic diseases. The ability of some NRs for binding ligands makes them potential pharmaceutical targets. Accordingly, certain liganded NRs have one or more cognate natural or synthetic ligands which are used in therapy. Their successes as drug targets are highlighted by the commonly used of retinoic acid for RAR α (NR1B1) (target for acute promyelocytic leukemia therapy), the synthetic antagonist tamoxifen for ER α (NR3A1) (target for breast cancer therapy), dexamethasone for GR (target for inflammatory disease treatment), or thiazolidinediones for PPAR γ (NR1C3) (target for treatment of type II diabetes).

NR pharmacology, compared with other receptor systems, has to a certain extent led the way in the appreciation that ligands may exert very diverse pharmacology, based on their individual chemical structure and the allosteric changes induced in the receptor/accessory protein complex. This can lead to very selective pharmacological effects, which may not necessarily be predicted from the experience with other agonists/►partial agonists/antagonists. Moreover, one of the most important mechanistic aspects that we have learnt over recent years is that the response of a given tissue is dictated by the set of coregulators with which NRs interact following ligand-induced allosteric alterations that generate, expose, or remove interaction surfaces. Therefore, chemistry can generate not only receptor-selective and various types of full, partial, and ►inverse agonists, but also molecules that activate only a subset of the functions induced by the cognate ligand or compounds that act in a cell-type selective manner, as the selective nuclear receptor modulators (►SNuRMs), leading to NR-based drug development.

Thus, and because therapeutic effects should be maximized and side effects minimized, the characterization of nuclear receptors and their associated proteins and the ligands which interact with them will remain a challenge to pharmacologists.

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Nucleic Acid Vaccination

►Genetic Vaccination

Nucleophilic

A nucleophilic compound or a group is attracted by a nuclei and has the tendency to share or donate electrons.

Nucleosome

The nucleosome represents the first level of DNA condensation and is the basic building block of all chromatin structures. It was discovered in 1973 and consists of a central histone octamer with about 150 base pairs of DNA wrapped around.

►Histone Acetylation

Nucleus Accumbens

The nucleus accumbens is part of the limbic system. It receives dopaminergic input through the mesolimbic system that originates from cell bodies in the ventral segmental area (A10 cell group). This mesolimbic dopaminergic pathway is part of the reward pathways. Drugs of abuse (cocaine, amphetamine, opiates or nicotine) have been shown to increase the level of dopamine release in these neurons.

►Drug Addiction/Dependence

Obesity

A disorder defined by an excess amount of white adipose tissue such that health is compromised. It is generally defined on the basis of body mass index (BMI) which is calculated as weight in kg/height in m². Normal weight is regarded as a BMI between 18.5 and 24.9, overweight as 25 and 29.9 and obesity as ≥ 30 . These figures are based on Europeans and North Americans, but in some other populations, such as in East Asia, the BMI threshold for obesity is increasingly considered to be lower.

- ▶ Adipokines
- ▶ Antiobesity Drugs

leads to oedema in nephrotic syndrome and malnutrition. Increased capillary permeability is responsible for oedema in inflammation and burns.

- ▶ Diuretics

Oestrogens

- ▶ Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

OCT

- ▶ Organic Cation Transporters

Off-resin Analysis

Off-resin analysis is analysis of a compound cleaved off a polymeric carrier material, usually in solution.

- ▶ Combinatorial Chemistry

Oedema

Oedema refers to an accumulation of interstitial fluid to a point where it is palpable or visible. In general this point is reached with a fluid volume of 2–3 liters. Oedema formation is the result of a shift of fluid into the interstitial space due to primary disturbances in the hydraulic forces governing transcapillary fluid transport and of subsequent excessive fluid reabsorption by the kidneys. Deranged capillary hydraulic pressures initiate oedema formation in congestive heart failure, and liver cirrhosis whereas a deranged plasma oncotic pressure

Olfactory Epithelium

The epithelium covering the nasal cavity. This epithelium contains numerous cell types including the specialized olfactory sensory neurons which detect the chemical stimuli derived from smells by a specific family of G protein-coupled receptors known as olfactory receptors.

- ▶ Trace Amines

Olfactory Receptors

Sensory receptors that structurally and functionally belong to the G protein coupled receptor superfamily. Olfactory receptors are a large GPCR family with >300 members in human that are expressed in neurons of the nasal olfactory epithelium where they sense mostly volatile olfactory molecule. The overall number of olfactory receptors differs widely between species and an expansion of different receptors is in particular obvious in species that depend on their olfactory sense for survival.

► Orphan Receptors

Oligonucleotide

A short (typically up to 80 nucleotides) single-strand DNA molecule.

► Microarray Technology

Oligopeptidase

An endopeptidase that is incapable of cleaving proteins but can cleave small peptides. An example is thimet oligopeptidase (*MEROPS* M03.001).

► Non-viral Peptidases

Omega Peptidase

An exopeptidase that does not cleave standard peptide bonds. An example is pyroglutamyl-peptidase I (*MEROPS* C15.010), which releases an N-terminal pyroglutamyl from hormones such as thyrotropin-releasing hormone and luteinizing hormone. Omega peptidases are included in Enzyme Nomenclature subclass 3.4.19.

► Non-viral Peptidases

On-resin Analysis

On-resin analysis is analysis of compound attached to a polymeric carrier material.

► Combinatorial Chemistry

Oncogenes

Oncogenes are genes, which confer malignancy on a cell. Proto-oncogenes are genes that normally control cell division and differentiation; however, they can be converted to oncogenes. Oncogenes often affect one or more signal transduction mechanisms, which impact on the cell division machinery.

- RNA Interference (RNAi) – siRNA
- Proto-oncogenes
- Targeted Cancer Therapy
- Cancer (Molecular Mechanisms of Therapy)

Oncogenesis

Oncogenesis is the process of cancer initiation; the term is essentially synonymous with carcinogenesis.

► Cancer (Molecular Mechanisms of Therapy)

Ontology

The word ontology has a long history in philosophy, in which it refers to the study of being as such. In information science, an ontology is an explicit formal specification of how to represent the objects, concepts, and other entities that are assumed to exist in some area of interest and the relationships among them.

► Bioinformatics

Open Reading Frame

Synonym

ORF

Definition

An opening frame contains a series of codons (base triplets) coding for amino acids without any termination codons. There are six potential reading frames of an unidentified sequence.

- Bioinformatics
- Microarray Technology

Opiates

In the strict sense, opiates are drugs which are derived from opium and include the natural products morphine, codeine, thebaine and many semi-synthetic congeners derived from them. In the wider sense, opiates are morphine-like drugs with non-peptidic structures. The old term opiates is now more and more replaced by the term opioids which applies to any substance, whether endogenous or synthetic, peptidic or non-peptidic, that produces morphine-like effects through an action on opioid receptors.

- Opioid System

Opioid Receptors

- Opioid Systems

σ -Opioid Receptors

σ -Opioid receptors are postulated receptors, which mediate the “dysphoric” effects (anxiety, hallucinations, bad dreams etc.), which are produced by some opioids. They are not true opioid receptors, as many other drugs also interact with them. Of the opioids, only benzomorphans, such as pentazocine, interact with sigma-receptors. The molecular identity of sigma-receptors is not known.

Opioid Systems

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Synonyms

Opioid receptors; Endogenous opioid peptides; Nociceptin/orphanin FQ; ORL-1

Definition

Opioid systems comprise opiate alkaloids and the families of ►endogenous opioid peptides (β -endorphin, enkephalins, and dynorphins) interacting with the three opioid receptor subtypes (μ , δ , κ). Pharmacological conventions define “opioid” when the action is produced by a prototypic opiate drug such as morphine and is antagonized by the antagonist naloxone. The classification of the three opioid receptors was confirmed by cloning of the three opioid receptor genes based on the fact that they share a homology of more than 60%. Interestingly, ►ORL-1 an orphan receptor exhibits the same high degree of similarity to the opioid receptors. However, classical opioids do not bind to this receptor, and the endogenous ligand discovered for ORL-1 the peptide nociceptin or orphanin FQ (N/OFQ) causes actions which are different from that of the classical opioid peptides and which are not antagonized by naloxone. Nevertheless, based on the high degree of structural similarity nociceptin/OFQ and the corresponding ORL-1 receptor are regarded to belong to the opioid systems.

Basic Characteristics

During the last 25 years it became clear that there are three types of pharmacologically well-defined opioid receptors (μ , δ , κ) that belong to the heptahelical group of G-protein coupled receptors. The genes of these receptors show a more than 60% homology to each other. A further “orphan” receptor sharing a similarly high degree of homology to the classical opioid receptors was recently identified and named ORL-1 (“opioid receptor like”) (1; Table 1). The NC-IUPHAR Subcommittee on Opioid Receptors suggested the terms MOP for μ opioid, DOP for δ opioid, KOP for κ opioid (KOP), and NOP for ORL-1 receptors [1].

The opioid receptors are targets of a large variety of exogenous (drugs) and endogenous ligands. Beginning with the discovery of the enkephalins in 1975, several endogenous opioid peptides have been isolated that derive from three precursor genes pro-opiomelanocortin (POMC), pro-enkephalin, and prodynorphin (Table 2). β -endorphin derives from POMC. Besides

Opioid Systems. Table 1 Opioid receptors and ligands

Receptor subtype	μ (MOP)	δ (DOP)	κ (KOP)	ORL-1 (NOP)
Prototypic ligands	Morphine	Met/leu-enkephalin	Ethylketocyclazocine	Nociceptin/OFQ
Endogenous ligand	Endomorphin-1,-2	Met/leu-enkephalin	Dynorphin A	Nociceptin/OFQ
Selective agonists	DAMGO	DPDPE, D-ala ² -deltorphin II	Enadoline, U-50488	
Selective antagonists	(naloxone) CTAP	Naltrindole	Nor-binaltorphimine	J-113 397

Opioid Systems. Table 2 Endogenous opioid peptides

Proopiomelanocortin	Proenkephalin	Prodynorphin	Pronociceptin/ OFQ	(Proendomorphin)
β -endorphin	Met-enkephalin, leu-enkephalin, MERF, MERGL	Dynorphin A, dynorphin A(1–8), dynorphin B, α -neoendorphin, β -neoendorphin	Nociceptin/ OFQ	Endomorphin-1, endomorphin-2

the pentapeptides met-enkephalin (ME) and leu-enkephalins (LE), proenkephalin is processed in the heptapeptide met-enkephalin-arg⁶-phe⁷ (MERF) and the octapeptide met-enkephalin-arg⁶-gly⁷-leu⁸ (MERGL). Prodynorphin is processed into dynorphin A, dynorphin A (1–8), dynorphin B, α -, β -neoendorphin [2]. In addition, two endogenous pentapeptides have been isolated that exhibit a high selectivity for μ -opioid receptors (MOPs) and have been called endomorphin-1 and endomorphin-2. Attempts to clone the genes for the corresponding precursor molecules have not been successful.

The MOP is the classical target for morphine and mediates the analgesic and addictive affects of the drug [2]. Therefore, in MOP-deficient mice morphine does not exhibit analgesic and positive reinforcing properties [3]. The human MOP gene encodes a heptahelical protein of about 400 amino acids and is localized on chromosome 6q24-25. Of the endogenous peptides, β -endorphin and to a lesser extent also the enkephalins and dynorphins have affinities for MOP. Endomorphin-1 and 2 are two pentapeptides that show the highest selectivity for this receptor. Another selective agonist is the synthetic peptide DAMGO (D-Ala², nMe-Phe⁴, Gly⁵-ol) enkephalin). The action of MOP agonists are competitively blocked by the antagonist naloxone, which is not absolutely specific for MOPs. The somatostatin analogue CTAP (D-Phe-Cys-Thr-D-Trp-Arg-Thr-Pen-Thr-NH₂) has been found to be a more selective MOP antagonist. β -FNA (β -funaltrexamine) and naloxonazine are irreversible antagonists.

Several splice variants of MOP (formerly MOR-1) have been cloned (MOP-1A to MOR-1X). The B, C, and D variants differ in their amino acid sequence at the C-terminal end [4]. These receptor variants differ in their distribution in the central nervous system and in the rate of internalization and desensitization upon

agonist exposure but have similar binding and coupling properties.

The existence of further alternative transcripts of MOP was postulated by the observation that in knockout mice with disrupted exon 1, heroin but not morphine was still analgesically active. Based on earlier observations that the antagonist naloxazone blocked morphine-induced antinociception but not morphine-induced respiratory depression, a subdivision of the MOP in μ_1 and μ_2 was proposed. However, no discrete mRNA for each of these MOP subtypes has been found. It is, however, possible that subtypes of MOPs result from heterodimerization with other opioid receptors or by interaction with other proteins.

Moreover, there exist polymorphic MOP variants. An Asn40Asp polymorphism has been found with a high abundance in the Caucasian and Asian population. This receptor variant is less expressed in the brain and carrier of this polymorphism appears to need more opioids for analgesic treatment. There are many additional MOP polymorphisms with unknown functional significance. In spite of many studies there appears to exist no significant association of polymorphisms in the MOP gene and drug addiction [5].

Highest concentrations of MOPs are found in the thalamus, caudate, neocortex in the brain, but the receptors are also present in gastrointestinal tract, immune cells, and other peripheral tissues.

The δ opioid receptor (DOP) is the primary target for met- and leu-enkephalin which also exhibits affinities for μ and κ receptors [2]. DPDPE (D-Pen², D-Pen⁵) enkephalin is a selective agonist, and naltrindole a selective antagonist on DOP. Only one DOP gene has been cloned to date. The human DOP is comprised of 372 amino acids and is localized on chromosome 1p34.3–36.1. Pharmacological experiments in rodents indicate the subdivision into δ_1 and δ_2 receptors.

However, currently no genes encoding these δ receptor isoforms have been cloned. In addition, there is no evidence for the existence of splice variants of this receptor. A DOP that lacks the third intracellular loop was generated by atypical mRNA processing in human melanoma tissues. This atypically processed receptor did not couple to G-proteins. There are several single nucleotide polymorphisms in the *DOP* gene. Associations studies did not reveal a significant relation between the polymorphisms in the *DOP* gene and drug addiction.

A high concentration of DOPs is found in the olfactory bulb, the neocortex, caudate putamen, and in the spinal cord, but they are also present in the gastrointestinal tract and other peripheral tissues. The functional roles of DOP are less clearly established than for MOP; they may have a role in analgesia, gastrointestinal motility, mood and behaviour as well as in cardiovascular regulation [2].

The KOP receptor is the natural target for prodynorphin-derived peptides, such as dynorphin A, dynorphin B, α -neoendorphin etc. The prototypical ligand is ethylketocyclazocine (EKC). Enadoline and U-50488 are selective agonists and norbinaltorphimine is an irreversible selective antagonist [2]. The human KOP gene encodes a protein of 380 amino acids which is localized on chromosome 8q11.12. From the binding characteristics of the prototypical ligand EKC, evidence for the subdivisions in κ_1 , κ_2 and κ_3 has been provided. However, there are no functional pharmacological data supporting these subdivisions. Moreover, no mRNAs coding for these receptor isoforms have been identified. Furthermore, in mice deficient in μ , δ and KOP receptors ("triple knockouts") no evidence for κ_2 binding sites could be found. A KOP with an alternative start site and with no known functional significance has been identified. There exist several polymorphisms in the *KOP* gene. Interestingly, recent studies show a significant association of single nucleotide polymorphisms of KOP (and also for PDYN) with opioid and alcohol addiction.

High concentrations of KOP have been found in the cerebral cortex and hypothalamus; KOP is also present in the gastrointestinal tract, in immune cells as well as in other peripheral tissues. KOPs have been implicated in the regulation of nociception, diuresis, feeding, neuro-endocrine and immune system functions [2].

The ORL-1 receptor was identified by its high homology to the other opioid receptor subtypes and termed "opioid receptor like". However, none of the endogenous opioid peptides or the opiate drugs shows a high affinity for this receptor. An endogenous ligand that binds to ORL-1 with high affinity has been identified and termed N/OFQ. Recently, J-113397, a drug with potent and selective antagonist activity at ORL-1 receptors, has been characterized.

According to the NC-IUPHAR Subcommittee on Opioid Receptors it was proposed to term ORL-1 receptor as NOP receptor [1]. The human NOP receptor gene encodes a protein of 370 amino acids. Splice variants have been found in the human and mouse NOP receptor with no known functional significance. NOP receptors are widely distributed throughout the brain and in the spinal cord. They are also present in immune cells. A functional role for N/OFQ has been proposed in nociception, locomotoric activity, reward, stress, and immunomodulation.

The OP group of receptors share common effector mechanisms. All receptors couple via pertussis toxin-sensitive Go and Gi proteins leading to: (i) inhibition of adenylate cyclase; (ii) reduction of Ca^{2+} currents via diverse Ca^{2+} channels; (iii) activation of inward rectifying K^+ channels. In addition, the majority of these receptors cause the activation of phospholipase A_2 (PLA_2), phospholipase $\text{C}\beta$ ($\text{PLC}\beta$), phospholipase D_2 and of MAP (mitogen-activated protein) kinase (Table 3).

Tolerance develops in response to continuous opioid agonist treatment. At the cellular level, receptor desensitization is caused by phosphorylation of receptor kinases leading to uncoupling of the receptor from the G-proteins. Binding of β -arrestin to the phosphorylated receptor initiates receptor endocytosis, which is followed by receptor dephosphorylation and recycling to the membrane. A part of the receptors is degraded in lysosomes. Opioids differ in their ability to internalize opioid receptors. For instance, morphine and buprenorphine are unable to internalize receptors, whereas methadone and fentanyl induce a substantial receptor endocytosis. Since internalization followed by recycling causes a resensitization of the receptors, methadone and fentanyl show a lower development of tolerance. An additional explanation for the differential development of tolerance by opioids is based on the theory of spare receptors. Opioids with a lower intrinsic activity, such as morphine or the partial agonist buprenorphine have to occupy a larger fraction of opioid receptors to produce the same effect. These opioids show therefore a faster tolerance development than opioids with a higher number of spare receptors (i.e., fentanyl or methadone). Moreover, adaptive mechanisms which antagonize opioid action can influence the development of tolerance. Thus, chronic administration of μ opioids has been shown to cause a

Opioid Systems. Table 3 Cellular responses to opioids

Inhibition of adenylate cyclase	Activation of PLA_2
Inhibition of voltage operated potassium channels	Activation of $\text{PLC}\beta$
	Activation of PLD_2
Activation of inwardly rectifying potassium channels	Activation of MAP kinase

super activation of certain subtypes of adenylate cyclase. In this case higher doses of opioids are required to inhibit the super activated adenylate cyclase. Long-term changes in adenylate cyclase activity result in activation of cAMP response element binding protein (CREB). This may result in changes in the expression of genes involved in opioid addiction.

In addition to the MOP, DOP, KOP, and NOP receptors the existence of several other opioid receptors has been proposed. The σ receptor, originally classified as an opioid receptor, is no longer regarded as such, since naloxone does not act as an antagonist at this receptor. The σ receptor rather appears to be the target of phencyclidine and related drugs. A σ receptor that does not have the heptahelical structure of G-protein coupled receptor has been recently cloned. A receptor with selective avidity for β -endorphin has been proposed and termed ϵ receptor. In addition, a so-called λ receptor was postulated on the basis of binding experiments. Recently, a ζ opioid receptor was cloned that binds Met-enkephalin in a naloxone displaceable manner and is proposed to regulate cell growth. The relationship of the ζ receptor, which shares no sequence homology to the OP receptors, awaits elucidation.

There has been an extensive search for additional opioid receptor genes with homology to μ , δ , and κ receptors which was, however, unsuccessful. It is likely, therefore, that the functional properties of the subdivision of μ , δ , and κ receptors as well as that of the ϵ and λ receptors results from alternate mRNA processing, posttranslational modification of the receptor, and/or from the formation of homo- and heterodimeric receptor complexes.

Drugs

Of the μ agonists (Table 3) morphine is the classical opioid alkaloid clinically used for treatment of pain. Codeine is a further naturally occurring derivative of morphine with a methylated phenolic hydroxyl group. Its potency is about 10% that of morphine. It is converted to morphine by the CYP2D6 enzyme system. In individuals with gene mutation in the CYP2D6 enzyme system (about 10% of the Caucasian population) codeine shows a marked reduced analgesic potency. Undesirable effects of morphine such as respiratory depression, development of tolerance/dependence led to search for analogues. A first semisynthetic morphine derivative was diacetyl morphine (heroin) which, however, was soon shown to exhibit a higher addictive liability than morphine. Modification of the morphine structure resulted in the development of other semisynthetic morphine derivatives, such as hydromorphone and oxycodone. A simplification of the morphine structure led to the development of fully synthetic drugs such as pethidine, methadone, fentanyl,

sufentanil, alfentanil, and remifentanil. These opiates differ in their potencies and/or pharmacokinetics, but exhibit similar side effects as morphine. Pethidine has a lower potency than morphine (about 20%). It is metabolized to norpethidine which can accumulate and induce convulsions. Therefore, pethidine should be used only to treat acute pain. In view of its spasmolytic effect it is indicated to treat pain caused by kidney stones. The long-acting methadone is a substitute for heroin in addicted patients. Fentanyl and its short-acting and potent derivatives are generally used during anaesthesia. In addition, fentanyl is increasingly used as a plaster preparation (transdermal therapeutic system) for treatment of patients with chronic pain. This allows the maintenance of therapeutic fentanyl level over a period of 3 days with less obstipatory side effects. In general, for the treatment of chronic pain long-acting preparations of opioid drugs (see Table 4) are preferentially used to minimize the development of opioid addiction. Other structural modifications led to the development of the oripavine derivatives (e.g. etorphine and buprenorphine).

Etorphine is much more potent than morphine and its catatonic action is used for sedating large animals. Buprenorphine is a long-acting partial agonist at μ opioid receptors which shows a ceiling effect for inducing analgesia in animals. In humans it is an effective analgesic and is also applied as a plaster to treat chronic pain. Moreover, buprenorphine is also used as a substitute for heroin in addicts. The μ opioid antagonists (naloxone and naltrexone) are clinically given for treatment of heroin overdose. In addition, the longer-acting naltrexone is used for preventing relapse in former heroin addicts. The short-acting antagonist naloxone is also used in combination with tilidine (Valoron®). After oral consumption tilidine is metabolized in the liver to the opioid active nortilidine, whereas the antagonist naloxone is rapidly degraded. The addition of naloxone prevents the intravenous abuse of the preparation by heroin addicts. Recently, naloxone has also been used as a long-acting oral preparation

Opioid Systems. Table 4 Opioid Drugs

Agonists	Partial agonists	Antagonists
Alfentanil, codein, dihydromorphone, etorphine, fentanyl, heroin, hydromorphone, levomethadone, morphine, oxycodone, pethidine, piritramide, remifentanyl, sufentanil, tilidine, tramadol	Buprenorphine, pentazocine	Naloxone, naltrexone

together with oxycodone (Tarwin®). The continuous absorption of naloxone by the gut decreases the obstipatory side effect of the opioid agonist oxycodone. Tramadol is a weak opioid agonist. It is a racemic mixture. The (+) enantiomer which has a very low affinity for the opioid receptor is metabolized to the more active opioid *O*-desmethytramadol. The (–) enantiomer inhibits the reuptake of serotonin and noradrenalin. These monoamines mediate the descending inhibition of pain transmission in the spinal cord. Codeine, tramadol, and tilidine/naloxone are weak opioids, which are applied in stage 2 of the pain scale of the WHO. For treatment of severe pain (stage 3) strong opioids such as morphine, buprenorphine, hydromorphone, or oxycodone are used.

κ receptor agonists produce a powerful antinociceptive effect and do not substitute for morphine in dependent animals. In addition, κ receptor agonists have neuroprotective effects in animal models of cerebral ischemia and traumatic head injury. However, clinical trials using κ agonists unmasked the dysphoric and psychotropic side effects of these drugs in humans. Pentazocin is a synthetic benzomorphan derivative: It is a mixed agonist/antagonist with κ receptor agonistic and partial agonistic properties on the μ receptor. Its interaction with κ receptors has been attributed to its hallucinogenic and dysphoric effects. Clinical trials are presently being carried out to explore whether κ agonists that do not readily penetrate the blood–brain barrier are useful in producing a peripheral analgesic effect in inflammatory pain.

Although preclinical studies suggest that δ agonists are potent analgesics with fewer side effects than μ agonists, none of the nonpeptide agonists or antagonists has been introduced into clinical investigations. Similarly, the clinical perspectives of the use of agonists and antagonists on the ORL-1 (NOP) receptor have to wait for the outcome of clinical trials.

- Analgesics
- Nociception
- Non-steroidal Anti-Inflammatory Drugs

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Opium

Opium is an extract of the juice of the poppy *Papaver somniferum*, which contains more than 20 distinct alkaloids, including morphine, codeine and papaverine.

- Opioid System

Oral Proteins

Pore-forming subunits of some forms of store-operated Ca^{2+} channels.

- IP_3 Receptors

Oral Anticoagulants

- Anticoagulants

Oral Blood Glucose Lowering Drugs

- Antidiabetic Drugs Other than Insulin

Oral Contraceptives

- Contraceptives

Oral Hypoglycaemic Agents

- Antidiabetic Drugs Other than Insulin

Orexigenic

Appetite-stimulating. Neuropeptide modulators and gut hormones with orexigenic effects are neuropeptide Y (NPY), agouti-related protein (AGRP), melanin-concentrating hormone (MCH), endocannabinoids, galanin, ghrelin and others.

► Anti-Obesity Drugs

Orexins

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Synonyms

Orexin A (OX-A) and Orexin-B (OX-B); Hypocretin-1 (Hcrt1) and Hypocretin-2 (Hcrt2)

Definition

Orexin-A (OX-A) and Orexin-B (OX-B) are ►**bioactive peptides** derived from a common precursor gene. Both peptides bind with differential affinity to two ►**Gq/11-protein coupled receptors** (OX1R, OX2R) in numerous targets throughout and even outside the nervous system of many species. In mammals, orexins are almost exclusively expressed in a small subset of ►**hypothalamic** neurons integrating a variety of intero- and exteroceptive, ►**nutritional-metabolic** and ►**circadian**-photoc influences. Orexins exert direct neuroexcitatory effects on ►**noradrenergic**, ►**histaminergic**, ►**dopaminergic**, ►**serotonergic**, ►**cholinergic**, and ►**GABAergic** cell populations. Thereby the orexin system drives glutamate transmission and whole brain activity to maintain systemic ►**bistability** and synchronize basic body functions. Loss of orexin neurons causes ►**narcolepsy-cataplexy**. Hyperactivity may predispose to ►**addiction**, as orexins link ►**appetite**, ►**arousal**, and neuroendocrine control with memory functions.

Basic Characteristics

Orexins were discovered in 1998, independently by two scientific groups using reverse genetics [1] and pharmacology [2]. The name orexin (from Greek: orexis = appetite) points to a systemic function first attributed to orexins: Control of ►**food intake** and ►**energy metabolism** (expenditure) [2, 3]. Conserved homologies with members of the *incretin*-peptide family and their

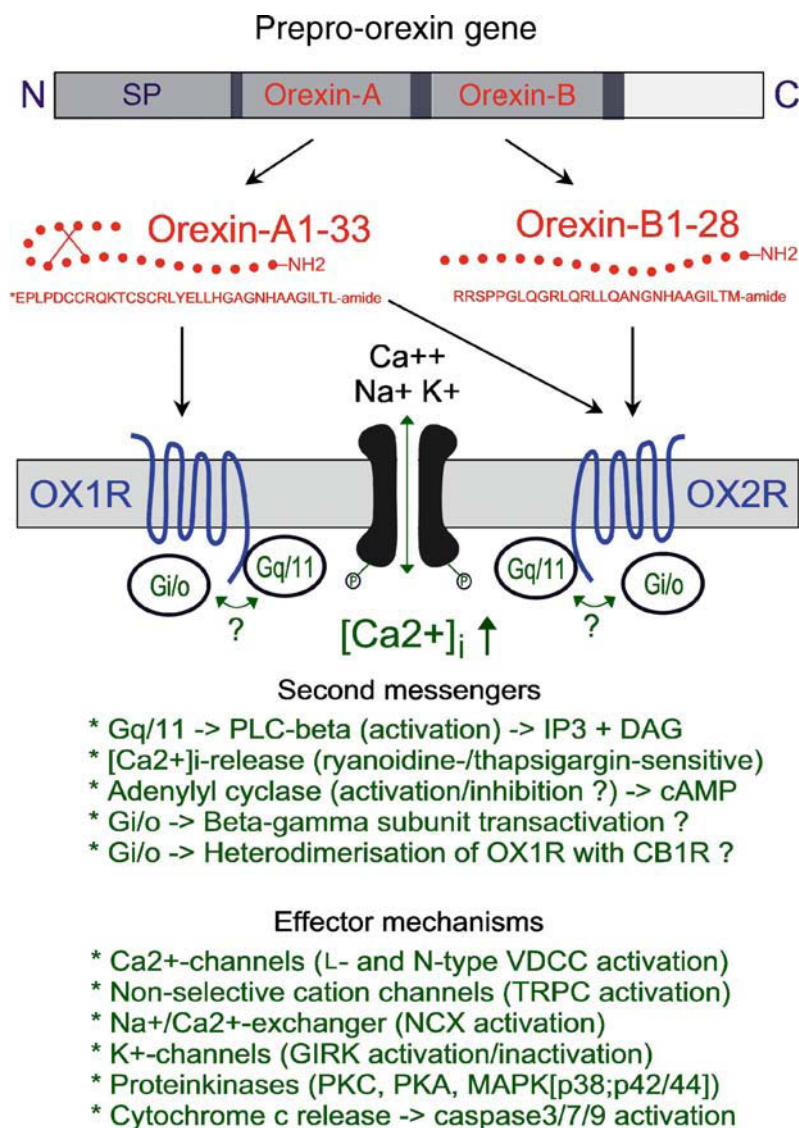
almost exclusive hypothalamic origin in mammals prompted for the synonym hypocretins (=“hypothalamic secretins”, Hcrt1, and Hcrt2, respectively) [1]. Association with narcolepsy-cataplexy [4, 5] indicates their major function: Control of ►**behavioral state** and systemic ►**bistability**. This includes consolidated states of sleep and wakefulness, mood, cognition, and movement, all of which are associated with aminergic transmission and compromised in narcolepsy-cataplexy. Orexins antagonize sleep and muscle atonia promoting arousal and postural stability, particularly in the context of consummatory behaviors. In the meantime, a plethora of additional orexinergic functions have been delineated [1–5]: ►**sympathoadrenal** and ►**neuroendocrine** outflow, ►**feeding rhythm**, ►**thermoregulation**, and ►**learning and memory**, all of which ultimately subserve behavioral state stability, ►**energy homeostasis** and survival. The pleiotropy of orexins is likely relevant for many disorders of ►**sleep**, ►**eating**, ►**mood**, ►**movement**, and ►**memory**.

Molecular Prerequisites of the Orexin System

The orexin system constitutes three phylogenetically conserved genes (Fig. 1). One encodes for the precursor peptide preproorexin (ENSG00000161610), a member of the incretin (PACAP-VIP-glucagon-GLP-1-secretin) family of peptides that is highly conserved across species. Two separate other genes encode for the Gq/11-protein coupled orexin receptors OX1R and OX2R.

The biologically active peptides OX-A (*EPLPDC-CRQKTCSCRLYELLHGAGNHAAGILT-*amide*, 33 amino acids, 3,562 kDa) and OX-B (RPGPPGLQGR-LQRLQANGNHAAGILTM-*amide*, 28 amino acids; 2,937 kDa, 46% homology to OX-A) are C-terminally ►**RF-*amide* peptides** with approximately 50% sequence identity and slight bombesin-like sequence homology. They are derived from alternate splicing (convertase) and posttranslational modifications (cleavage). OX-A contains two sets of disulfide bonds playing a key role in receptor activation and prolonged half-life, compared to OX-B. The promoter region of the precursor gene contains ►**interferon** (INF α) response elements. Both mRNA and peptide expression in rodents arise by embryonic day E18 and start to diminish after 1 year of age.

Orexin receptors (OX1R and OX2R) are highly conserved (95%) across species. OX2Rs shares 64% identity with OX1Rs, but exhibit considerable differences with respect to agonist affinities, signal transduction and distribution throughout and even outside the nervous system. OX1Rs exhibit about 10–100-fold higher affinity to OX-A than OX-B, while OX2Rs bind both peptides with equal affinity. Binding of orexins to both receptors may be accomplished with [¹²⁵I]-orexin A and is rather specific, although binding of OX-B to ►**orphan G protein-coupled receptors** sensitive to other RF-*amide* peptides (neuropeptide FF) has been reported.



Orexins. Figure 1 Molecular basis of the orexin system (scheme). OX1R, OX2R: orexin receptor 1 and 2, CB1R: cannabinoid receptor 1, PLC: phospholipase C, IP3: inositol-3-phosphate, cAMP: cyclic-adenosine-monophosphate, VDCC: voltage-dependent calcium channel, TRPC: transient receptor potential channel, NCX: sodium calcium exchanger, GIRK: G protein-coupled inwardly rectifying potassium channel.

Studies in heterologous expression systems indicate that both OX1R and OX2R belong to the Gq/11-subclass of heteromeric G-proteins, commonly promoting Ca²⁺-entry and [Ca²⁺]_i-mobilization through coupling to IP3- and DAG/PKC-dependent signal transduction pathways. OX2Rs may also couple to Gi/o. Studies in native tissues indicate a higher degree of complexity in the signal transduction of OXRs. This includes activation of ▶voltage-dependent Ca²⁺ channels (VDCC), non-selective cationic and ▶transient receptor potential channels (TRPC), electrogenic ▶Na⁺/Ca²⁺ exchangers (NCX), and inhibition of previously activated G protein-coupled inwardly rectifying potassium channels

▶(GIRKs), thapsigargin- and cAMP-sensitive ▶PKA, and DAG-dependent ▶PKC activation, activation of ▶MAPK (p38, p42/44), ▶mTOR, protein synthesis, and proapoptotic pathways (cytochrome *c* release, caspase-3/7/9 activation). OX1Rs hypersensitize due to hetero-oligomerization with ▶endocannabinoid CB1 receptors, providing functional convergence of orexin and endocannabinoid signaling.

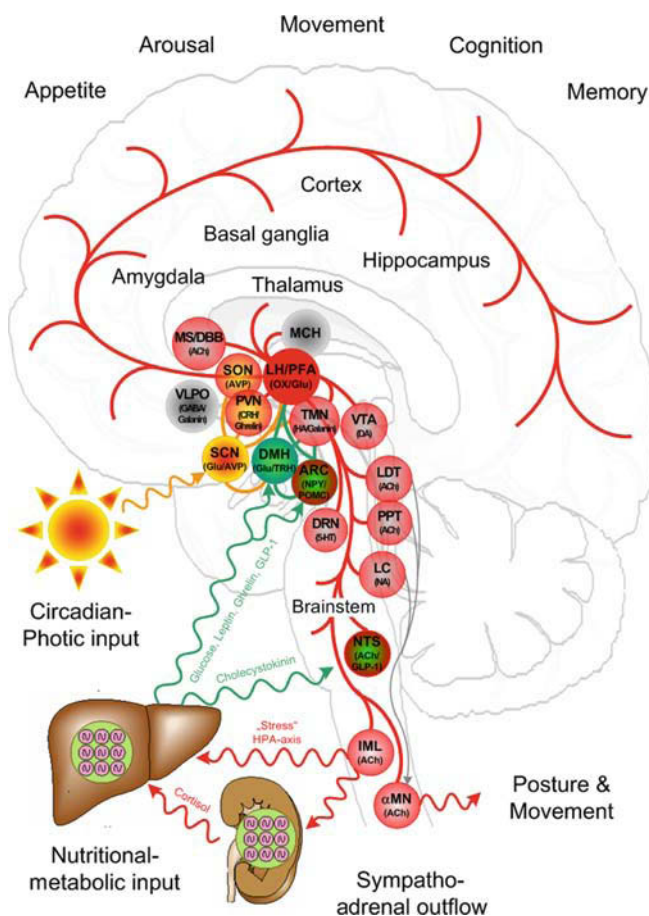
Functional Anatomy of the Orexin System

In the mammalian brain orexins are almost exclusively expressed in a small group of neurons located in the lateral ▶hypothalamus (LH) and perifornical area (PFA)

from where they project widely throughout the entire neuraxis (Fig. 2). The human brain contains about 50000–80000 orexin neurons. A similar restricted expression and widespread distribution of orexinergic projections and OXRs have been detected in monkey, hamster, cat, sheep, pig, chicken, various amphibians, and zebrafish.

Major efferent projections of the hypothalamic orexin system comprise descending and ascending, dorsal and ventral pathways that terminate preferentially in aminergic, endocrine, and autonomic control centers in the hypothalamus, ►midbrain, ►brainstem, and ►spinal cord, as well as in ►limbic cortical and subcortical structures, including ►septum, ►amygdala, ►thalamus,

nucleus accumbens, and ►hippocampus. Although a large proportion of orexin neurons contribute projections to multiple destinations, subgroups of cells with differential sensitivity to psychoactive drugs in the LH and PFA make contact with particular targets, suggesting functional dichotomy with respect to appetite and arousal control. The distribution of orexins and their receptors matches with the central representations of the autonomic nervous system, which form the major output for homeostatic and circadian control of peripheral organs. Indeed, 50% of the putative command neurons integrating somatomotor and cardiovascular functions have been identified as orexinergic. The most



Orexins. Figure 2 Functional anatomy of the orexin system with its major in- and output pathways. Hypothalamic orexin neurons (LH/PFA, red dot) convey circadian-photoc, nutritional-metabolic neuroendocrine input (yellow and green lines/dots, respectively) to wake-active aminergic, cholinergic, and cortical targets (red lines/dots). During sleep and satiety orexin neurons are inhibited by GABAergic inputs from the VLPO and other inhibitory influences (grey lines and dots). PFA: perifornical area, LH: lateral hypothalamus, OX: orexin, VLPO: ventrolateral preoptic area, MCH: melanin-concentrating hormone, NPY: neuropeptide Y, POMC: proopiomelanocortin, ARC: arcuate nucleus, TRH: thyrotropin-releasing hormone, DMH: dorsomedial hypothalamus, AVP: arginine-vasopressin, SCN: suprachiasmatic nucleus, OXY: oxytocin, SON: supraoptic nucleus, CRF: corticotropin-releasing factor, PVN: paraventricular nucleus, HA: histamine, TMN: tuberomammillary nucleus, NA: noradrenalin, LC: locus coeruleus, 5-HT: 5-hydroxy-tryptophan, DR: dorsal raphe, DA: dopamine, VTA: ventral tegmental area, SN: substantia nigra, Ach: acetylcholine, PPT/LDT: pedunculopontine/laterodorsal tegmentum, MS/DBB: medial septum/dorsal band of broca, GLP-1: glucagon-like-peptide-1, NTS: nucleus tractus solitarius, IML: intermediolateral tract, HPA: hypothalamo-pituitary-adrenal axis, αMN: alpha-motoneuron.

prominent extrahypothalamic connections of orexin neurons exists with aminergic and GABAergic cell groups in the locus coeruleus (LC, ►noradrenaline), ►dorsal raphe (DR, ►serotonin), ventral tegmental area/substantia nigra (VTA/SN, ►dopamine), and ►cholinergic neurons in the ►pedunculopontine/laterodorsal tegmentum (PPT/LDT), ►medial septum/dorsal band of broca (MS/DBB), ►parasympathetic (GLP-1 expressing) ►nucleus tractus solitarii (NTS), ►sympathetic intermediolateral tract (IML), and ►α-motoneurons (α-MN). Several hypothalamic and extrahypothalamic nuclei are reciprocally directly or indirectly interconnected with orexin neurons (Fig 2): (i) Histamine neurons in the ►tuberomammillary nucleus (TMN) and perifornical area (PFA) are intermingled with orexin neurons together with (ii) GABAergic afferents from the ►ventrolateral preoptic area (VLPO) coexpressing the neuroprotective peptide galanin. This triangle forms a bistable functional switch that controls maintenance of wakefulness and sleep–wake transitions and is a prominent target for sedative effects of GABAergic ►general anesthetics (e.g., propofol). Interestingly, both, the effects of orexins on arousal, as well as those of leptin and ghrelin on feeding depend on histamine H1R-function, suggesting that the histamine system has a key position in behavioral state and neuroendocrine outflow control. (iii) ►Melanin-concentrating hormone (MCH) neurons are another cell population spatially intermingled with orexin neurons in the lateral hypothalamus (LH) but exhibiting differential topological distribution and largely antagonistic or complementary functions. (iv) Orexigenic ►neuropeptide Y (NPY) and anorexigenic ►pro-opiomelanocortin (POMC) containing neurons in the ►arcuate nucleus (ARC) are integral part of a brain–gut axis that controls ►energy balance and ►food intake. (v) ►Thyrotropin-releasing hormone (TRH) coexpressing neurons in the ►paraventricular nucleus (PVN) together with (vi) ►arginine-vasopressin (AVP) positive neurons in the ►suprachiasmatic nucleus (SCN) convey neurohumoral nutritional-metabolic (relayed through the ARC) and circadian-photoc influences (relayed through retino-hypothalamic melanopsin-pathways to the SCN) to the ►dorsomedial hypothalamus (DMH). This DMH is a food-entrainable oscillator (Neural networks integrating circadian-photoc and nutritional-metabolic influences to produce rhythmic food anticipatory behavioural activity (FAA). In mammals, probably controlled by neurons in the dorsomedial in the hypothalamus (DMH).)controlling appetite and feeding-related circadian behaviors. Excitatory interactions of orexins with oxytocin (OXY)- and AVP-expressing neurons in the ►supraoptic nucleus (SON) affect fluid homeostasis, and possibly social and reproductive competence. Finally, ►corticotropin-releasing factor (CRF) expressing neurons in the ►paraventricular nucleus (PVN) mutually interact with orexins along all branches of the hypothalamo–pituitary–adrenal (HPA) axis, supporting energy expenditure in

contexts of increased demand during arousal and defense responses, but also stress-related behaviors (grooming) and stress-induced reinstatement of drugs of addiction, such as cocaine.

The diffuse nature of orexinergic projections is consistent with combined patterns of expression of the two orexin receptors, which is widespread but differential and often complementary within and even outside the CNS. OX2Rs are found primarily in structures attributed to control of sleep–wake functions, whereas OX1Rs are more abundant in ►limbic structures controlling aspects of feeding, autonomic outflow, ►energy homeostasis, and to a lesser extent also ►REM-sleep.

Extrahypothalamic OX-B-like immunoreactivity, reminiscent to that of CRF, has been described in clustered GABAergic neuronal populations, in the lateral division of central nucleus of the ►amygdala, the ►bed nucleus of the stria terminalis, and in the ►hippocampus. Moreover, ectopic expression of preproorexin mRNA in the gut, ependymal cells, neuroblastomas, and of orexin receptors in ►adrenal gland, cancer and ►hematopoietic stem cells suggests yet unexplored roles of orexins as paracrine factors controlling blood–brain barrier, and tumor or stem cell function.

Physiology and Function of the Orexin System

The activity of hypothalamic orexin neurons exhibits diurnal rhythms and varies according to behavioral and nutritional state. In vivo, orexin neurons fire spontaneously during active waking in association with movement when muscle tone is high. Activity is lower during quiet waking and absent during sleep when postural tone is low. In REM sleep, with minimal ►postural tone, orexin neurons are silent in spite of the muscle twitches occurring during this state. In vitro, orexin neurons display a rather low membrane potential (~ -50 mV) and activity is dynamically regulated by their intrinsic properties as well as excitatory feed forward and inhibitory feedback through a variety of transmitters and intero- and exteroceptive neuroendocrine, circadian (SCN, DMH), and gastrointestinal, nutritional-metabolic (DMH, ARC, NTS) state cues. Importantly, orexin neurons are excited by ►hypoglycemia and directly inhibited by ►glucose (through TASK, tandem-pore domain potassium channels). Likewise, ►ghrelin, a short-term hunger signal from the stomach excites orexin neurons and promotes food intake, while the adipostatic and long-term satiety factor ►leptin has inhibitory effects on orexinergic activity, energy expenditure, and food intake. Moreover, glutamatergic input to orexin neurons varies according to nutritional state in a leptin-sensitive manner, increasing with overnight food deprivation and decreasing by refeeding.

Orexin neurons, likely to be glutamatergic themselves, express the ►excitatory amino acid transporter EAAT3, ►vesicular glutamate transporters VGLUT1 and VGLUT2, secretogranin II, ionotropic (NMDAR,

AMPA) and metabotropic (mGluRs) ►glutamate receptors, and the neuronal ►pentraxin Narp, implicated in clustering of AMPA receptors. Other proteins detected in orexin neurons include precursor-protein convertase and ►dynorphin which modulates orexinergic signaling and plays a role in orexin-induced feeding, the transcription factor Stat-3, and receptors for GABA (epsilon subunit), biogenic amines, ►purines (ATP P2X, adenosin), opioids (mu and kappa), leptin, and ►pancreatic polypeptide Y4. Accordingly, intrinsic activity of orexin neurons (in vitro) is increased by glutamate, ATP, ►acetylcholine (M₃), ►ghrelin (GHSR), ►glucagon-like peptide-1 (GLP-1), CRF, AVP and OXY(V_{1A}), ►neurotensin, and ►cholecystokinin (CCK-A) but inhibited via negative feedback through presynaptic metabotropic glutamate autoreceptors (mGluRs), GABA_{A/B}, catecholamine (α_2), serotonin (5HT_{1A}), ►leptin (ob-R), NPY (Y1), MCH, and sleep-promoting, anti-epileptic effects of ►adenosine (A₁).

Pathophysiology and Dysfunction of the Orexin System

Hypofunction of the orexin system is causally related to the sleep disorder narcolepsy-cataplexy, and animal models of that disease. In humans, loss and degeneration of orexin neurons, likely due to an ►autoimmune attack or ►neurotoxicity pathogenesis, is the most frequent (1:2000) cause of narcolepsy-cataplexy, a disease characterized by intrusions of REM sleep like phenomena into wakefulness, increased daytime sleepiness, sleep fragmentation, vivid dreaming, hypnagogic hallucinations, and in some cases cataplexy. Cataplexy is a sudden loss of postural muscle tone generally triggered by emotionally arousing, appetitive or pleasurable stimuli, such as joy and laughter in humans, or food presentation in animals. Interestingly, ►narcolepsy exhibits a high comorbidity with migraine, consistent with a role of orexins in nociception and pain, and possibly neuroinflammation.

Dogs with a sporadic mutation of OX2R trafficking and function, as well as mice with a complete destruction of orexin neurons (by expression of an orexin-ataxin transgene), or deletion of OX1/2-receptors exhibit a phenotype similar to that of narcolepsy-cataplexy in humans, including abnormalities in sleep architecture (decreased sleep onset REM latency, rapid intrusions of REM sleep phenomena into wakefulness), energy balance (increased ►body mass index), and neuroendocrine functions. Orexin-deficient mice eat less, but gain weight, and show disrupted ►food anticipatory behavior (in face of restricted daily feeding), indicating imbalanced energy homeostasis and feeding rhythms, associated with reduced expression of clock genes (*Per1*, *Bmal1*, and *NPAS2*). Likewise, lesions of the suprachiasmatic nucleus or loss of ►clock gene function eliminates diurnal orexin rhythms and is associated with attenuated diurnal feeding rhythms, hyperphagia, ►obesity, and a ►metabolic

syndrome comprising hyperleptinemia, hyperlipidemia, hepatic steatosis, hyperglycemia, and hypoinsulinemia. Overexpression of ►uncoupling protein-2 (UCP-2) selectively in mitochondria of orexin neurons in mice increased ►life span and decreased ►core body temperature through local warming of ambient hypothalamic structures. This emphasizes a central role of orexins in energy balance and ►thermoregulation, independent from peripheral thermogenesis.

Hyperactivity of the orexin system, e.g. triggered by energy depletion, metabolic failure, hypoglycemia or hypoxia, in the context of starvation, sleep deprivation, and stress, may predispose to ►addiction and ►compulsion. Orexin neurons are selectively activated by natural rewards such as food (a common trigger of canine narcolepsy) but also by withdrawal from drugs of abuse, such as ►morphine and ►cocaine. Moreover, orexins through mechanisms promoting synaptic plasticity control reinforcement of hippocampal long-term potentiation and memory consolidation. In contrast, ►narcoleptics rarely develop ►addiction, in spite of the addictive properties of most of their pharmacological treatments. Likewise, orexin-deficient mice are less susceptible to drugs of addiction and show attenuated ►stress responses, behaviorally (defense) and in sympathoadrenal functions (blood pressure and heart rate). Pleiotropy of orexinergic dysfunction may thus be relevant for many disorders of eating, sleep, mood, movement, and memory.

Drugs

Pharmacological targeting of the orexin receptors by selective OX ►agonists or ►antagonists could provide near causal therapy of narcolepsy-cataplexy, or efficient treatment of ►insomnia, and possibly addiction, respectively. However, orally active, blood-brain barrier permeable, small molecule OX agonists are not yet available. All drugs currently used to treat symptoms of narcolepsy-cataplexy thus mimic orexinergic effects by interfering with aminergic (amphetamines, antidepressants, modafinil) and GABAergic (gamma-hydroxy butyrate) neurotransmission. Of the available prodrugs antagonizing orexin receptor function only ACT-078573 has been tested in humans, so far (Table 1). ACT-078573 is an orally active, brain penetrating OX1/2R-antagonist and has been introduced as an efficient sleep-promoting and possibly anti-addictive drug in rats, dogs, and humans. It lacks unwanted side effects of GABAergic sedative substances (i.e., REM sleep suppression and addiction) and did not produce cataplexy in humans after acute ingestion. This suggests that other factors produced by orexin neurons (e.g., ►NARP, dynorphin) may contribute to the development of cataplexy, or chronic blockade of OX1/2R-antagonists may be required to produce cataplexy. Long-term studies in humans are

Orexins. Table 1 Pharmacology of orexin receptors

Nomenclature	OX ₁	OX ₂
Other names	Orexin receptor type 1	Orexin receptor type 2
Ensembl ID	► ENSG00000121764	► ENSG00000137252
Signaltransduction	G _{q/11}	G _{q/11}
Potency	Orexin-A > orexin-B	Orexin-A = orexin-B
Selective agonists	—	[Ala ¹¹ ,D-Leu ¹⁵]orexin-B
Selective antagonists	SB408124, SB334867A	—
Nonselective antagonists	ACT-078573*	ACT-078573 ^a

Abbreviations: SB334867A, 1-(2-methylbenzoxanzol-6-yl)-3-[1,5]naphthyridin-4-yl-urea hydrochloride; SB408124, 1-(6,8-difluoro-2-methyl-quinolin-4-yl)-3-(4-dimethylamino-phenyl)-urea;

^aACT-078573 (Brisbare-Roch et al. Nat Med, 2007)

necessary to prove the safety and tolerability of drugs that interfere with orexins. This is of particular importance facing the pleiotropic actions of orexins on cardiovascular and neuroendocrine functions.

Alternate ways to interfere with the orexin system may be via inhibition of ►dipeptidyl peptidases or proteolysis-resistant peptide analogs as shown for other peptides. This could prolong and boost orexinergic signaling. OX-A but not OX-B can enter the brain by simple diffusion via the ►blood–brain barrier. Abundance of orexins and their receptors in the olfactory bulb and throughout all parts of the central ►olfactory system may offer transnasal routes for drug application.

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Organic Cations

Organic cations are compounds that meet the two criteria of a single positive charge (no negative charge) and a significant degree of hydrophobicity (alkyl chains or aromatic rings, little capacity to form hydrogen

bonds). Typical examples are tetraethylammonium (TEA), N¹-methylnicotinamide (NMN), and MPP⁺.

►Organic Cation Transporters (OCT)

Organic Nitrates

Organic nitrates are polyol esters of nitric acid. This group of drugs includes glyceryl trinitrate (nitroglycerine), isosorbide mononitrate (ISMN), isosorbide dinitrate (ISDN) and amyl nitrite (AN). Organic nitrates release nitric oxide (NO), a process which involves an enzymatic step. NO release from organic nitrates activates the soluble form of guanylyl cyclase by interacting with a heme group in the enzyme. The formation of cyclic cGMP by guanylyl cyclase leads then to the relaxation of smooth muscle cells. Organic nitrates at clinical concentrations mainly lead to a relaxation of venous capacitance vessels. Organic nitrates are mainly used for the prophylaxis or acute treatment of anginal pain. The antianginal effect of organic nitrates decreases after repeated administration (tolerance). The main adverse effects are hypotension and headache.

►Guanylyl Cyclases

Organophosphates

Organophosphates are a group of pentavalent phosphorus compounds, which contain an organic group (e.g. parathion, malathion, tabun, sarin, soman).

Organophosphates are irreversible acetylcholinesterase inhibitors. They are used as insecticides or “nerve gas”

► **Acetylcholinesterase**

ORL-1

The ORL-1 receptor was identified by its high homology to the other opioid receptor subtypes and termed “opioid receptor like”, although none of the endogenous peptides or opiate drugs show a high affinity for this receptor. An endogenous peptide which binds to this “orphan” receptor with high affinity was identified and termed nocepin or orphanin FQ.

► **Opioid Systems**

Orphan G-protein-coupled Receptors

► **Orphan Receptors**

Orphan Nuclear Receptors

Orphan nuclear receptors are a sub-group of the nuclear receptor (NR) family of transcription factors. The nuclear receptors (NRs) are characterized by: (i) a common modular domain structure and (ii) direct binding to DNA via zinc-finger motifs. In general, the modular domain structure consists of the following: an N-terminal domain containing a ligand-independent activation function (constitutive activity); the DNA-binding domain (DBD) containing two zinc-finger motifs; the hinge domain which links the DBD and the ligand-binding domain (LBD); and the LBD containing the ligand binding site and ligand-dependent activation function (ligand-dependent activity). Orphan receptors share the structural characteristics of NRs, including the presence of a LBD, though ligands for this sub-group of NRs have not been identified to date.

- **Peroxisome Proliferator-Activated Receptors (PPARs)**
- **P450 Mono-oxygenase System**
- **Nuclear Receptors**
- **Orphan Receptors**

Orphan Receptors

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Synonyms

Orphan G protein-coupled receptors; Orphan GPCRs; Orphan serpentine receptors; Orphan nuclear receptor

Definition

Orphan receptors are proteins that bind and are activated by hitherto unknown signaling molecules (called ligands, neurotransmitters, or hormones). However, they share structural components with identified receptors whose signaling molecules are already known. The physiological functions of orphan receptors are unidentified. The identification of the natural ligand of an orphan receptor holds the key to our understanding of its physiological role and potential as a drug target.

Basic Mechanism

Members of two structurally very distinct receptor families are termed orphan receptors. One is the family of the ► **nuclear receptors** which are cytosolic proteins that upon ligand binding change their conformation, translocate to nuclear binding sites, and act as transcription factors to regulate the expression of other genes. The second group of receptor proteins is the ► **G protein coupled receptor** (GPCR) superfamily representing seven-transmembrane-helix surface receptors that reside in the plasma membrane. When bound to a specific ligand, conformational changes in the receptor molecule lead to activation of heterotrimeric G proteins (short for guanine nucleotide binding proteins) which in turn act as signal transducers to switch on their respective effector proteins such as ► **phospholipases**, ► **adenylyl cyclases**, or ion channels. This essay addresses in particular orphan GPCRs, but the basic principles described below for the identification of ligands also applies to nuclear orphan receptors.

Historically, orphan receptors were first identified by homology screening techniques based on low-stringency DNA hybridization followed by PCR-assisted strategies. The success of these methods led to the realization that large numbers of GPCRs exist but gave still just a glimpse of the overall size of this receptor superfamily. With the completion of the human genome sequencing project, ► **bioinformatics** search tools quickly led to a comprehensive picture of the receptor repertoire in the human genome. Presently the genes of about 800 human GPCRs have been identified from which approximately 50% are represented by ► **olfactory** and ► **taste receptors**. The other half consists

of receptors with already known ligands leaving presently about 110 orphan GPCRs. As primary step toward drug discovery, determining a physiological function for these newly identified receptors is of vital importance, and thus identification of a natural or ►**surrogate ligand(s)** is primary aim. Orphan receptor specific ligands can be directly injected into animals and serve as indispensable molecular tools to study the *in vivo* function of the orphan receptor by acutely altering animal behavior and physiology, hence shedding light on the receptor's intrinsic function. In order to identify ligands, there are at least two necessary steps to complete. First the development of a suitable assay system and secondly the screening of synthetic compound libraries, that is, compilations of biological active ligands or biological tissue extracts that contain molecules able to activate a particular orphan receptor. The whole process is generally referred to as ►**reverse pharmacology** and interchangeably used with the term deorphanization.

Assay Systems for Orphan Receptors

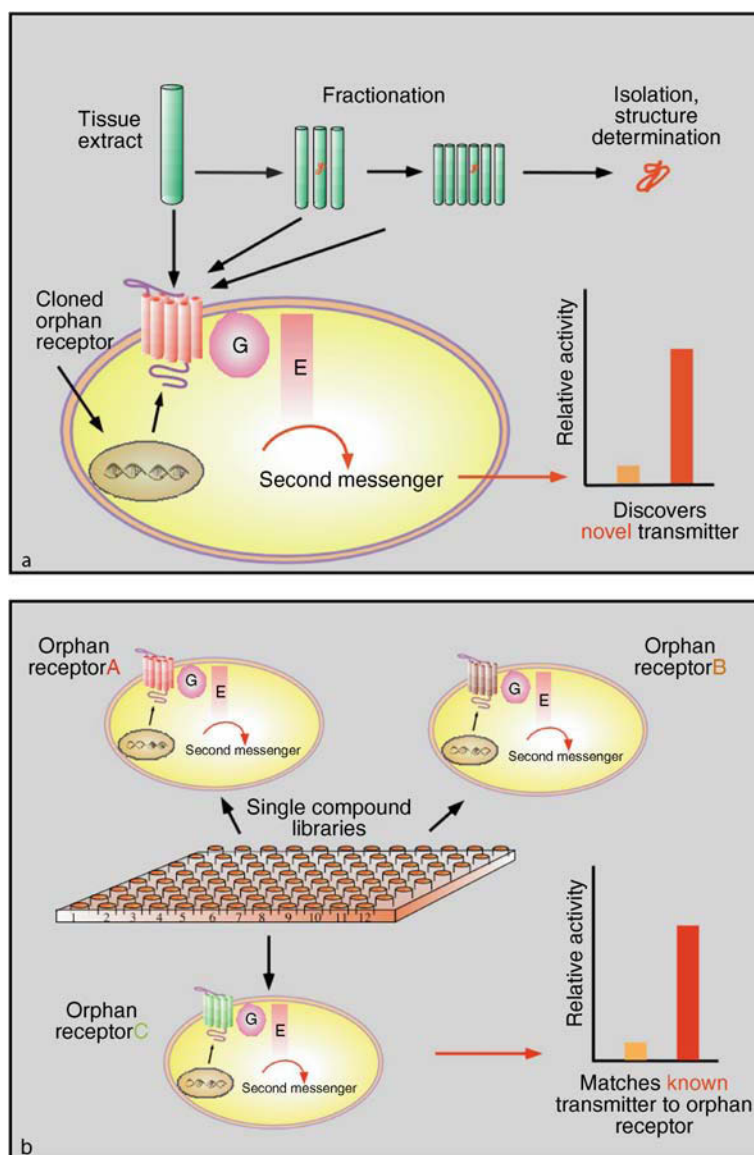
Orphan receptors, in particular the orphan GPCRs, rely on an intact cellular environment for proper function that provides the necessary G protein and effector machinery to enable the identification of an activating ligand (Fig. 1). The receptors are initially cloned by recombinant DNA technologies and then introduced into a suitable expression system, most often immortalized mammalian cell lines. These cells are capable of expressing the receptor at the cell surface and in addition provide all the additional protein components to couple the receptor to a signal transduction pathway. In the most direct way, the orphan receptor expressing cells can be directly probed for specific binding of radioactively labeled ligand(s). Such binding strategies proved highly successful when applied to orphans at the beginning of the orphan receptor era, a time many potential neurotransmitters were known but their molecular targets remained elusive. However, the binding approach is limited to already known ligand structures. The much more exciting, but also more difficult approach, is to utilize the orphan receptors functional properties to isolate natural ligands, which means to identify novel cellular transmitters. Since the binding of a ligand to the orphan will lead to conformational changes in the receptor protein, this in turn generates a downstream signaling event that can be readily detected by physicochemical methods. With such a strategy, the orphan receptor itself serves as a sensor for its own unknown ligand(s).

The majority of GPCRs couple to three families of G protein alpha subunits which differ in their ability to activate three distinct primary signaling cascades. The stimulatory G-protein G_{α_s} , positively regulates the activity of adenylyl cyclase causing an increase of intracellular cAMP levels as second messenger. In

contrast, G_{α_i} protein subunits inhibit adenylyl cyclase, hence lowering intracellular cAMP levels. G proteins of the $G_{\alpha_{q/11}}$ type activate phospholipase C β leading to transient increase of intracellular calcium that in turn activates many cellular processes. By directly measuring either changes in intracellular cAMP or increase of intracellular calcium levels the activity of the majority of orphan GPCRs can be monitored. However, until recently there was no structural analysis method able to predict which second messenger will be modulated by a given orphan receptor, forcing the experimenter to run different assay systems simultaneously. ►**Constitutive receptor activity** can be used to predict the likely signaling mechanism and promiscuous $G_{\alpha_{15/16}}$ subunits or genetically engineered G protein chimeras can be used to force the coupling of an orphan GPCRs to a desired effector system, reducing the number of assays significantly. In addition, a plethora of alternative assay systems for GPCRs are presently available that can be adapted for monitoring orphan receptors. Finally it has to be stressed out that an orphan receptor assay system, due to the lack of an agonist is difficult to validate and therefore some uncertainty about the functionality of the system always remains. Despite the odds, 5–10 receptors are successfully deorphanized each year since 1999.

Ligand Screening for Orphan Receptors

Once an assay system has been established the search for ligands can be initiated. Screening is a rather elaborate task and is preferentially done in ►**high throughput screening** assay formats to handle the enormous number of assay points that are necessary to find novel ligands. Since ligands for GPCRs are extremely diverse in structure and function the right selection of candidate ligand libraries is of paramount importance. Pharmaceutical companies compiled sets of known and putative ligands for screening which contain proven GPCR small molecules, lipids, candidate molecules, and metabolites from the literature, peptides and proteins predicted by ►**bioinformatics**. Screening all libraries on all orphan receptors can be an expensive endeavor. To limit the cost of such a venture, bioinformatics can help to classify orphan GPCRs based on their sequence similarity and to predict candidate ligands. Such an approach pairs candidate ligand libraries to the most likely receptor structure and increases the chance to match the right ligand with its potential receptor. So far, ligand matching proved to be the most successful approach of all, but its limits are obvious. Technically the most challenging but also the one that generates most impact on basic sciences is the identification of novel ligands from biological tissue extracts. In this approach candidate tissues, generally the one where the orphan receptor is highly expressed, are homogenized and extracted. The biological extracts are then fractionated by biochemical methods and screened for biological activity using the same assays methods as described above. Following



Orphan Receptors. Figure 1 Two principal strategies to identify molecules that activate orphan receptor as exemplified by the orphan G protein coupled receptors. (a) A cloned receptor is introduced into a mammalian cell line where it is expressed at the cell surface. The cells are exposed to minute amounts of tissue extracts and then immediately monitored for changes in intracellular second messenger molecules, like cyclic adenosine monophosphate or inositol 1,4,5-trisphosphate. If the extract contains an orphan receptor specific molecule, the relative activity of the cell expressing the orphan receptor will be significantly higher compared to that of a control cell line. Positive tissue extracts are repeatedly fractionated and tested on the orphan receptor cell, till the active compound is sufficiently pure for structure determination. This strategy discovers novel signaling molecules. (b) An alternative approach is to use libraries of single compounds that are already known for their biological activity. These libraries are systematically applied to different orphan receptors expressed in mammalian cells and the relative responses monitored. If a single compound matches a certain orphan receptor, its host cell line will specifically respond but others remain silent. This strategy can only identify already known biologically active molecules. G, G-protein; E, Effector protein (see text).

the detection of a biological activity, it is necessary to purify the active compound by repetitive fractionations and finally elucidate the structure. This approach was so far most successful for the identification of novel

peptides where minute amounts of material can be analyzed due to the existence of highly sensitive analytical technology. Although this approach faces many technical challenges, identification of novel

transmitter belonging to other chemical classes than peptides is expected in the future.

Assays for Orphan Receptors without Ligands

Numerous examples of successful identification of novel ligands for orphan receptors exist, but recently discovered and unexpected GPCR phenomena unfold the possibility that some orphan receptors might act as regulators of other GPCRs, thus having ligand-independent functions. A large body of evidence suggests that some GPCRs are capable of forming both homo- and heterodimers in the cellular membrane that is required for proper receptor trafficking. Formation of heterodimers might also result in a changed pharmacology and even generate new ligand binding sites. The remaining “difficult” orphans are candidates for the discovery of novel GPCR functions and will need the development of new assay systems that are capable of probing beyond classical GPCR signal transduction, for example, protein–protein interaction, receptor trafficking, and novel signaling pathways.

Pharmacological Relevance

GPCRs are important drug targets. About 30% of all clinically marketed drugs are modulators of GPCR function making GPCRs the most successful of any target class relevant to drug discovery. Orphan receptors belonging to the GPCR superfamily are indeed viewed as potential new drug targets and are currently being exploited for their potential in treating diseases, including obesity, sleep-related and mental disorders, inflammation, and cardiovascular disease. Ligand identification for orphan receptors will lead to the discovery of novel response systems and open new therapeutic avenues for a variety of human diseases. Each identified receptor ligand pair in itself provides an assay system that can be directly applied to ►[drug discovery](#), i.e. sieving through chemical libraries that contain hundreds of thousands of small molecules with the goal to identify so-called hit compounds acting as agonist or antagonist at the screened receptor system. Hit compounds can be further refined by medicinal chemistry and developed into therapeutic drugs depending on their toxicological, pharmacokinetic, metabolic profiles as well as their patentability. However, drug development programs are costly endeavors and investment into novel receptor–ligand pairs demands their validation as potential drug targets. Presently, the major challenge lies in the identification of the physiological and pathophysiological importance of newly deorphaned receptors in order to motivate drug development.

There are now many examples of novel peptide transmitter systems that have been identified through orphan receptor ligand screening and greatly impacted the basic understanding of human physiology. Prime examples for the discovery of novel transmitter with therapeutic implications are the neuropeptides ►[orphaninFQ/](#)

[nociceptin](#), ►[orexin/hypocretin](#), and ►[ghrelin](#) for treatment of anxiety, sleep disorders, and obesity, respectively. Other orphan GPCRs turned out to be the pharmaceutical targets of already known therapeutic drugs. For example, the oral available and potent ►[antiplatelet drug](#) clopidogrel was found to bind an orphan GPCR whose natural ligand was identified as adenosine diphosphate (ADP) and now named P2Y₁₂. The identification of this therapeutically proven orphan receptor made it an immediate candidate for drug development without further validation. Since the entire number of biochemical targets that have led to marketed drugs is fewer than 500 and the GPCRs make up the majority of those, it is anticipated that at least some novel therapeutic drugs will emerge from orphan receptor research.

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Orphan Serpentine Receptors

►Orphan Receptors

OrphaninFQ/Nociceptin

A 17 amino acid long peptide sequentially related to opioid peptides in particular dynorphin A. OFQ/N is inactive at the δ , κ , and μ opioid receptors, but binds to its own NOP receptor (formerly ORL-1, for opioid receptor like-1). In contrast to opioid peptides, OFQ/N has no direct analgesic properties. OFQ/N is the first example for the discovery of a novel neurotransmitter from tissue extracts by using an orphan receptor as bait. Centrally administered in rodents, OFQ/N exerts anxiolytic properties. OFQ/N agonists and antagonists

are presently undergoing assessment for their usefulness to treat various mental diseases, including depression and anorexia nervosa.

- ▶ Phospholipases
- ▶ Orphan Receptors
- ▶ Opioid System

Osmotic Laxatives

- ▶ Laxatives

Osteoarthritis

Osteoarthritis is a disease of the load-bearing joints, characterised by gradual erosion of cartilage and deformation of bone. Pain is the main symptom, initially eased by rest, but later analgesics such as aspirin-like drugs are indicated.

- ▶ Cyclooxygenases
- ▶ Non-steroidal Anti-inflammatory Drugs

Osteoblast

An osteoblast is the cell forming new bone. Osteoblasts are derived from stromal bone marrow stem cells.

- ▶ Bone Metabolism

Osteoclast

One of the cell types involved in bone metabolism. In concert with osteoblasts, their cellular counterpart, these cells maintain bone hemostasis and constant

blood calcium levels by regulating formation (osteoblasts) and resorption (osteoclasts) of mineralized bone matrix. Osteoclasts differentiate from myeloid precursors in the bone marrow.

- ▶ Bone Metabolism

Osteocyte

A cell embedded within the mineralised matrix of bone. Osteocytes are derived from former osteoblasts and are responsible for intra-skeletal sensing and signalling.

- ▶ Bone Metabolism

Osteoporosis

Osteoporosis is a common condition, in which bone density is decreased as a consequence of an imbalance between bone formation (osteoblast) and bone loss (osteoclast). This leads to fragile bones, which are at an increased risk for fractures. The term “porosis” means spongy, which describes the large holes seen in these bones.

- ▶ Bone Metabolism
- ▶ Glucocorticoids
- ▶ Tyrosine Kinases

Osteotropic Hormones

Systemic regulators of osteoblast, osteocyte and osteoclast functions, and therefore of bone metabolism. The major “bone-seeking” hormones are parathyroid hormone (PTH), 1,25-dihydroxyvitamin D3 (calcitriol) and the various sex hormones.

- ▶ Bone Metabolism

Ototoxicity

Ototoxicity describes a harmful effect on the inner ear, especially the sensory cells in the cochlea and the vestibular organ. Aminoglycosides are an example of drugs with ototoxic side effects.

► Ribosomal Protein Synthesis Inhibitors

Oxazolidinones

Oxazolidinones are a new class of synthetic antimicrobial agents, which have activity against many important pathogens, including methicillin-resistant *Staphylococcus aureus* and others. Oxazolidinones (e.g. linezolid or eperezolid) inhibit bacterial protein synthesis by inhibiting the formation of the 70S initiation complex by binding to the 50S ribosomal subunit close to the interface with the 30S subunit.

► Ribosomal Protein Synthesis Inhibitors

Oxidase, Mixed Function

► P450 Mono-oxygenase System

Oxidative Stress

Increased accumulation of reactive oxygen species (ROS) in biological systems.

- Reactive Oxygen Species
- Antioxidants
- Oxidative Stress

Oxytocin

► Vasopressin/Oxytocin

P1 Receptors

► Adenosine Receptors

p53

p53 is a tumor suppressor gene known to enhance apoptosis.

► Neurodegeneration ► Cancer

p70S6 Kinase

p70S6 kinase is a serine/threonine protein kinase, which is involved in the regulation of translation by phosphorylating the 40S ribosomal protein S6. Insulin and several growth factors activate the kinase by phosphorylation in a phosphatidylinositol (PI) 3-kinase-dependent and rapamycin-sensitive manner. Phosphorylation of S6 protein leads to the translation of mRNA with a characteristic 5' polypyrimidine sequence motif.

► Insulin Receptor

P450 Induction

The process whereby cellular and tissue levels of one or more cytochrome P450 enzymes are increased in response to treatment of cells, or a whole organism,

with certain drugs or environmental chemicals referred to as P450 inducers. P450 induction leads to an increase in the cell's capacity for P450-catalyzed oxidative metabolism of many xenochemicals, as well as endogenous steroidal and fatty acid P450 substrates.

► Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes

P450 Mono-oxygenase System

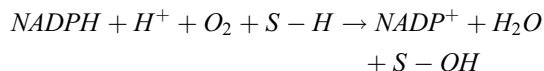
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Synonyms

Cytochrome P450 (CYP); Cytochrome P450 isozymes; Cytochrome P450 mono-oxygenases; Mixed function oxidases

Definition

Cytochrome P450 (CYP) mono-oxygenases, also called mixed function oxidases, are versatile hemoprotein enzymes that catalyze the cleavage of molecular oxygen to incorporate one oxygen atom into a substrate molecule and one atom into water [1]. The general stoichiometry of the reaction is as follows (S-H, substrate):



The numerous biotransformations catalyzed by cytochrome P450 enzymes include aromatic and aliphatic hydroxylations, epoxidations of olefinic and aromatic structures, oxidations and oxidative dealkylations of heteroatoms and as well as some reductive reactions. Cytochromes P450 of higher animals may be classified into two broad categories depending on whether their substrates are primarily endogenous or xenobiotic substances. Thus, CYP enzymes of families 1–3 catalyze

the ►**phase I metabolism** of most drugs and ►**xenobiotics**, often a prerequisite for a phase II conjugation reaction and subsequent elimination from the body. Although usually involved in detoxification, sometimes the products of CYP-catalyzed reactions can also be pharmacologically active (in this case the substrate is called a ►**prodrug**), or it may be carcinogenic, toxic, or otherwise harmful. In contrast, the CYP enzymes of families CYP4 to CYP51 primarily participate in critical physiological pathways leading to steroid hormones, bile acids, prostaglandins and other important endogenous compounds.

Basic Characteristics

Cytochrome P450 Electron Transport Systems

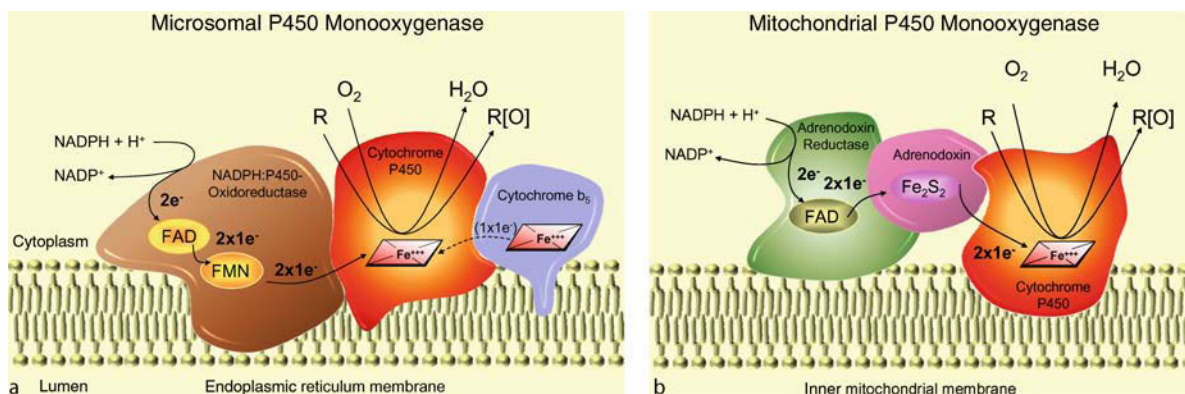
The P450 mono-oxygenase system functions as an electron transport chain in which electrons are transferred from cellular pyridine nucleotides (NADPH/NADH) to the P450 hemoprotein. In vertebrates, there are two principal types of P450s and electron transfer chains. One is found in the mitochondrial inner membrane and the other in the endoplasmic reticulum (ER). The electron donating protein of the ER system (Fig. 1a) is called NADPH-cytochrome P450 oxidoreductase (CYPOR). It consists of two domains with two different prosthetic flavin groups FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide), which transfer two electrons acquired from NADPH directly, but only one at a time, to the P450 heme iron. CYPOR is bound to the ER membrane by its N-terminal tail, whereas the bulk of the protein is on the cytosolic side of the ER membrane. In humans, CYPOR is encoded by a single gene (*POR*) on chromosome 7 which encodes a protein of 677 amino acids. The crystal structure of CYPOR has been determined. In certain reactions catalyzed by microsomal P450s, the second electron can also be donated by cytochrome b_5 , a smaller heme containing protein (MW ~ 15 kDa),

which accepts electrons from NADH via the flavoprotein NADH-cytochrome b_5 -reductase.

In mitochondria (Fig. 1b), the electron acceptor protein is also a flavoprotein termed NADPH-adrenodoxin reductase (MW ~ 50 kDa) because it was discovered in the adrenal cortex and because it donates its electrons not directly to the P450 but to the smaller redox protein adrenodoxin (MW ~ 12.5 kDa). The two iron-sulphur clusters of this protein serve as electron shuttle between the flavoprotein and the mitochondrial P450.

Cytochrome P450 Structure

The name cytochrome P450 (P for pigment) derives from the unusual spectrum compared to other hemoproteins. Carbon monoxide strongly binds to the reduced (ferrous) heme iron inducing a strong absorption at 450 nm that was first discovered in 1958 and that can be used to quantitate cytochrome P450 via its reduced CO-difference spectrum. The structural difference between cytochrome P450 and other hemoproteins is the unusual fifth ligand to the heme iron, which is a histidine in other hemoproteins and a conserved cysteine thiolate in all P450s, located close to their C-termini. X-ray crystal structures available not only for bacterial but also for several human microsomal P450s have confirmed the role of the conserved thiolate ligand and provided deep insights into substrate binding and structural peculiarities [2]. The many sequences available and the recent structural data indicate significant structural similarities between all P450s, which have about 500 amino acids and most likely evolved from a single common ancestor gene. Microsomal P450s are bound to the membrane by a single hydrophobic N-terminal transmembrane anchor in such an orientation that the heme moiety is colocalized with the CYPOR flavodomain at the cytoplasmic side of the ER. Mitochondrial P450s are synthesized as slightly larger precursors with an N-terminal signal sequence that is being cleaved



P450 Mono-oxygenase System. Figure 1 (a) Components of the microsomal P450 mono-oxygenase system. (b) Components of the mitochondrial P450 mono-oxygenase system.

off during translocation of the protein to the inner mitochondrial membrane.

Cytochrome P450 Gene Superfamily

Cytochromes P450 are the products of a gene superfamily with currently more than 6,000 known forms (isozymes) from all types of living organisms. Cytochrome P450 proteins are classified into families and subfamilies based on their sequence similarities. Any two forms with more than 40% identity at the amino acid level belong to the same family (indicated by an arabic numeral), whereas they belong to the same subfamily (indicated by a capital letter) if they share more than 55% of identical amino acids. Individual isozymes of the same subfamily are distinguished by an additional arabic number. Based on the results of the human genome project, humans have 57 individual functional *CYP* genes which are organized in 18 families and 43 subfamilies and which are found dispersed over all autosomal chromosomes. A particular aspect of mammalian cytochrome P450 systems is the high number of ►pseudogenes which harbour mutations that prevent the expression of functional proteins. Humans have more than 25 *CYP* pseudogenes that make molecular genetic analysis complex due to their high sequence homology. Other mammals share the same 18 *CYP* families with humans, but the number of individual functional isozymes in each family or subfamily can be very different. Based on their role in metabolism, the 18 *CYP* families can be broadly classified into those catalyzing the metabolism of xenobiotics (families *CYP1*, *CYP2*, and *CYP3*), and those that are responsible for biotransformations of important endogenous substances (families *CYP4* to *CYP51*; Table 1). Whereas mutations in several *CYP* genes of the first category are frequent in the population and lead to polymorphic drug oxidation, mutations in CYPs catalyzing physiological reactions are rare and often the cause for inherited metabolic disorders.

Human Drug Metabolizing Cytochromes P450

Typical characteristics of the drug metabolizing P450s are their broad and overlapping substrate specificities and their extremely variable expression and function both in terms of inter- and intraindividual variation which has a tremendous influence on the ►pharmacokinetics of most drugs in clinical use. The in vivo activity of a specific P450 enzyme can principally be estimated by measuring metabolite concentrations of an ingested selective probe drug in urine, blood or breath of patients. Three basic mechanisms are responsible for variability in the activity of drug metabolizing enzymes: (i) genetic polymorphism; (ii) gene regulatory mechanisms that lead to enzyme induction or downregulation; and (iii) direct inhibition of enzyme activity.

Polymorphisms in Drug Metabolizing P450s

A ►genetic polymorphism is a difference in DNA sequence that has a frequency of at least 1% in a population. Many drug-metabolizing enzyme genes are highly polymorphic with consequences for expression and function of the gene product, such that they affect the disposition of drugs and xenobiotics (►pharmacogenetics). The best studied example of a P450 genetic polymorphism is that of *CYP2D6* [4]. It results in two major phenotypes in the population, the extensive metabolizer (EM) phenotype and the ►poor metabolizer (PM) phenotype. The PM phenotype is inherited as an autosomal recessive trait and affects about 5–10% of Caucasians who are unable to metabolize a range of drugs which are substrates for CYP2D6. For drugs with narrow therapeutic window, these individuals may be at risk to develop adverse drug reactions when given normal drug doses. Further examples for polymorphisms in P450 genes are mentioned below. The Internet Home Page of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee lists all known human cytochrome P450 alleles at ►<http://www.cypalleles.ki.se>.

Gene Regulatory Mechanisms in Drug Metabolizing P450s

A variety of regulatory mechanisms are acting on different P450 genes to regulate their constitutive expression and response to environmental stimuli [5]. Members of the *CYP1* family and some other drug metabolizing enzymes including some UDP-glucuronosyltransferases (UGTs) are collectively induced by polycyclic aromatic hydrocarbons (PAH) that serve as ligands to a specialized receptor called the ►aryl hydrocarbon receptor (AhR) which translocates to the nucleus following binding of another protein component called arnt (AhR nuclear translocator). In the nucleus these two proteins bind DNA and activate transcription. Another regulatory mechanism is responsible for the 40–50 fold induction of *CYP2B* enzymes of humans and rats following administration of phenobarbital and other barbiturates. The ►orphan nuclear receptor CAR (constitutively activated receptor) plays a central role in mediating the effect of phenobarbital that leads to induction of CYP2B6. PXR, another orphan nuclear receptor, binds a different range of ligands including the antibiotic rifampin, and leads to induction of a different profile of genes, in particular CYP3A4. Both CAR and PXR bind to DNA in the form of heterodimers with the retinoic X-receptor, RXR, as binding partner [5]. Other chemicals also induce P450s, e.g. ethanol induces the CYP2E1 enzyme. The general feature of these regulatory mechanisms is that substrates induce their own metabolism.

Direct Inhibition of P450

Direct inhibition of P450 enzymatic activity is the most common reason for drug–drug interactions.

P450 Mono-oxygenase System. Table 1 Overview of the cytochrome P450 superfamily in humans

Family	Subfamily, genes, pseudogenes (P)							Typical substrates/functions
CYP 1	A1	A2	A8P					PAHs, PCBs, estrogens, aromatic amines
	B1							PAHs, PCBs, retinoids
CYP 2	A6	A7	A13	A18P				Nicotine, coumarin, nitrosamines
	B6	B7P						Cyclophosphamide, bupropion, efavirenz
	C8	C9	C18	C19	C58P	C62P		Taxol (C8), NSAIDS, warfarin (C9), omeprazole (C19)
	D6	D7P	D8P					Antidepressants, opioids, beta-blockers
	E1							Ethanol, halothane, acetone
	F1	F1P						Naphthalene, styrene
	G1P	G2P						No function
	J2							Arachidonic acid, ebastine hydroxylation
	R1							Vitamin D3 25-hydroxylase
	S1							All-trans retinoic acid, naphthalene
	T2P	T3P						No function
	U1							Arachidonic acid omega-hydroxylation
	W1							Bioactivation of procarcinogens
CYP 3	A4	A5	A5P	A7	A43			Cyclosporin, antidepressants, testosterone
CYP 4	A11	A22						Fatty acid omega-hydroxylation
	B1							Fatty acids, clofibrate, steroids
	F2	F3	F8	F9P	F10P	F11	F12	Arachidonic acid, leukotrienes, prostaglandines
		F22	F23P	F24P				
	V2							Fatty acid and steroid metabolism
	X1							Unknown function
	Z1	Z2P						Unknown function
CYP 5								Thromboxane A2 synthase
CYP 7	A1							Cholesterol 7- α hydroxylase ($\rightarrow$ bile acids)
	B1							Oxysterol 7- α -hydroxylase
CYP 8	A1							Prostacyclin synthase
	B1							Steroid 12- α hydroxylase ($\rightarrow$ cholic acid)
CYP 11	A1							Cholesterol side chain cleavage
	B1	B2						Steroid 11- β or 18 hydroxylase ($\rightarrow$ cortisol, aldosteron)
CYP 17								Steroid 17- α hydroxylase/17-20 lyase
CYP 19								Steroid aromatase ($\rightarrow$ estrogens)
CYP 20								Unknown function
CYP 21	A1P	A2						Steroid 21-hydroxylase
CYP 24								Vitamin D degradation (24-hydroxylase)
CYP 26	A1							All trans retinoic acid hydroxylase
	B1							Retinoic acid hydroxylase
	C1							Retinoic acid hydroxylase
CYP 27	A1							27-Hydroxylation in bile acid biosynthesis
	B1							Vitamin D3 1- α hydroxylase (kidney)
	C1							unknown function
CYP 39								24-Hydroxycholesterol 7-hydroxylase
CYP 46								Cholesterol 24-hydroxylase
CYP 51		51P1	51P2	51P3				Lanosterol 14- α demethylase (cholesterol biosynthesis)

Data mainly from reference [3].

P, pseudogenes; PAHs, polycyclic hydrocarbons; PCBs, polychlorinated biphenyls.

P450 inhibitors can be either of the competitive or of the mechanism-based type. Potent competitive inhibitors are often, but not always, substrates which have a high affinity for the enzyme. Mechanism-based or suicide enzyme inhibition occurs when a substrate is activated to a reactive intermediate that subsequently binds either to the P450 polypeptide or to the heme moiety thereby inactivating it irreversibly. Examples for both types of inhibitors are known for almost every drug-metabolizing P450. Substance interfering with P450 enzyme activity may also originate from food, e.g. grapefruit juice contains CYP3A4 inhibitors that have significant effects on in vivo drug concentrations lasting several days.

Drugs

Human Drug Metabolizing CYPs

CYP1A1: Typical substrates are polycyclic aromatic hydrocarbons (PAHs) like benzo(a)pyrene or methyl-cholanthrene. The carcinogenicity of these substances depends on their metabolic activation by CYP1A1. Some of the PAHs that induce CYP1 enzymes via the Ah receptor are found in cigarette smoke and charred food. CYP1A1 is expressed at very low levels in the liver of uninduced individuals but is found in extra-hepatic tissues including placenta, lung, and lymphocytes. **CYP1A2** has a broader substrate specificity than CYP1A1, including many aromatic and heterocyclic amines and is more abundantly expressed in human liver. Typical substrates are caffeine, phenacetine, clozapine, estrogens and others. Caffeine N3-demethylation can be used as a selective 1A2 marker activity, whereas 7-ethoxyresorufin O-deethylation or phenacetin O-deethylation reflect both CYP1A isozymes. Genetic polymorphisms have been found in all human CYP1 genes and their associations with various forms of cancer were intensely studied. The *CYP1A1/2* genes are located on chromosome 15.

CYP1B1 (chromosome 5) has been linked to primary congenital glaucoma. CYP1B1 is not regularly expressed in liver but is often found in various kinds of tumours. It metabolizes retinoids and many aromatic amines and PAHs to potentially carcinogenic products.

The CYP2 family is the largest CYP family in humans and it comprises about 16 functional genes and 11 pseudogenes.

CYP2A6 is the principal enzyme for nicotine metabolism. A selective probe drug for CYP2A6 is coumarin. Clinically important drug substrates are rare for this enzyme. Several genetic polymorphisms have been found in the *CYP2A6* gene which affect mainly expression levels. It has been suggested that smokers with genetically determined low CYP2A6 expression need to consume less nicotine to achieve the same satisfying blood levels of the drug.

CYP2B6 is the only functional isozyme of the 2B subfamily in humans, as *CYP2B7* is a pseudogene. Both genes are located within a large *CYP2* gene cluster on chromosome 19. CYP2B6 is highly variable owing to its inducibility by barbiturates and other drugs but its expression and function are also affected by frequent genetic polymorphisms. Clinically important substrates are the cytostatic cyclophosphamide, the antidepressant bupropion, and the antiretroviral drugs used in HIV treatment, efavirenz and nevirapine. S-Mephenytoin N-demethylation and bupropion hydroxylation are selective marker activities that can be used both in vitro and in vivo.

The **CYP2C** subfamily comprises the four genes *CYP2C8*, *2C9*, *2C18*, and *2C19*, which are together localized on chromosome 10. The members of this subfamily show surprisingly large variation in substrate specificity and regulation. **CYP2C8** appears to have a narrow substrate specificity with taxol 6- α hydroxylation being the most selective marker activity. **CYP2C9** is very abundantly expressed in human liver and has a broad substrate specificity accepting many weakly acidic substances like the hypoglycemic agent tolbutamide, the anticoagulant warfarin, the anticonvulsant phenytoin and several NSAIDs (nonsteroidal antiinflammatory drugs). **CYP2C19** substrates are (S)-mephenytoin, the 4-hydroxylation of which provides a very specific marker activity, the [proton pump inhibitor](#) omeprazole, the antimalarial proguanil, and diazepam. All *CYP2C* genes are genetically polymorphic. Clinically relevant are the two major variant alleles of *CYP2C9*, *2C9*2* and *2C9*3* which are associated with decreased enzyme activity, as well as the genetic polymorphism of *CYP2C19*, also known as the S-mephenytoin polymorphism, which affects about 3–5% of Caucasians and up to 20% of Asian populations in the homozygous form. **CYP2C18** was found to be expressed in liver only as mRNA but not as a protein.

CYP2D6 was the first P450 for which a classical pharmacogenetic polymorphism became known. The enzyme and its gene, which is localized together with two pseudogenes on chromosome 22, has been very thoroughly studied [4]. CYP2D6 is responsible for more than 70 different drug oxidations, mostly of substrates containing a basic nitrogen. They include antiarrhythmics (e.g. propafenone), antidepressants (e.g. amitriptyline, venlafaxine), antipsychotics (e.g. thioridazine), beta-Blockers (e.g. metoprolol), opioids (e.g. codeine) and more. Sparteine and debrisoquine, which are no longer in use, lead to the discovery of the genetic *CYP2D6* polymorphism, also known as the sparteine/debrisoquine polymorphism. More than 50 functionally distinct alleles which are associated with either complete lack of function (null-alleles) or with decreased or increased enzyme activity are known

(CYPallele nomenclature homepage at ►<http://www.cypalleles.ki.se>). The individual inherited allele combination (genotype) determines whether an individual will have the ►ultrarapid metabolizer (UM), extensive (EM), intermediate (IM) or ►poor metabolizer (PM) phenotype. About 5–10% of Caucasians carry two null-alleles and are consequently PMs; about 10–15% are IMs carrying alleles with reduced function, and about 10% are UMs some of whom carry an allele with a duplicated functional gene. In other ethnic populations, these percentages can be very different. Thus, in Asians the PM phenotype has a frequency of only 0.5–1%, whereas in certain Arabian and Eastern African populations, the frequency of the UM phenotype can be as high as 30%. The phenotype can be determined either by using one of several available specific probe drugs, e.g. dextrometorphan or metoprolol, or it can be predicted by genetic diagnosis.

CYP2E1 (chromosome 10) metabolizes small molecules including ethanol, halogenated hydrocarbons like halothane, as well as small aromatic and heterocyclic compounds, many of which also act as inducers. For example, it is known that CYP2E1 is induced in alcoholics. Only few drug substrates of CYP2E1 are known, but the enzyme activates many xenobiotic metabolites to toxic intermediates. Chlorzoxazone 6-hydroxylation has been proposed as a marker activity. Several polymorphisms were described in the *CYP2E1* gene, some of which were found to be more frequent in Asians and believed to be associated with increased cancer risk linked to smoking.

CYP2F1 appears to be expressed preferentially in lung where it bioactivates the selective pneumotoxins 3-methylindole and naphthalene.

CYP2J2 is abundant in cardiovascular tissue and active in the metabolism of arachidonic acid to eicosanoids that possess potent anti-inflammatory, vasodilatory, and fibrinolytic properties. Polymorphic alleles with reduced function are known. Some other *CYP2* subfamilies and isozymes listed in Table 1 are still not well characterized, in part because most of them were discovered in the course of the human genome project.

The **CYP3A** subfamily is highly important for human drug metabolism. CYP3A4 plays the major role because it is abundantly expressed not only in liver but also in intestinal enterocytes. CYP3A4 makes significant contributions to the metabolism of more than half of all clinically used drugs including large molecules like the immunosuppressant cyclosporin A, macrolide antibiotics like erythromycin, or anticancer drugs like taxol, and smaller molecules like benzodiazepines, HMGCoA reductase inhibitors, anaesthetics and many more. CYP3A4 expression levels are increased following exposure to a number of drugs that bind to the nuclear receptor PXR (pregnane X-receptor) which

increases the rate of *CYP3A4* gene transcription. Furthermore, there are sex-related differences in CYP3A4 expression with women having significantly higher levels, which translate into higher *in vivo* clearance of several drug substrates. Whereas genetic polymorphism does not appear to play a major role in determining CYP3A4 activity, expression of the two subfamily members, CYP3A5 and CYP3A7, is confined to a smaller fraction of the population who carry particular alleles of these genes. CYP3A7 is more abundantly expressed in fetal liver than in adult liver. CYP3A43 was found to be expressed at very low levels.

CYPs in Physiological Pathways

Most of these enzymes have steroids or fatty acids as their substrates (Table 1). Many P450s in endogenous biotransformation pathways are characterized by usually very narrow substrate and product specificity and by tight regulatory systems, especially those involved in steroid hormone biosynthesis.

The **CYP4** family comprises a larger number of subfamilies and isozymes. The major substrates for CYP4A forms are fatty acids which are hydroxylated at their omega position. The physiological significance of this is largely unknown. Non-fatty acid substrates may be metabolized by specific CYP4A forms. Expression of CYP4 enzymes is regulated by peroxisome proliferators like clofibrate (peroxisomes oxidize fatty acids), drugs that bind to another nuclear receptor termed PPAR (peroxisome proliferator activated receptor).

CYP5 synthesizes thromboxane A₂, a fatty acid in the arachidonic acid cascade that causes platelet aggregation. Aspirin prevents platelet aggregation because it blocks the ►cyclooxygenases COX1 and COX2 which catalyze the initial step of the biotransformation of arachidonic acid to thromboxane and prostaglandins.

CYP7A1 catalyzes the 7 α -hydroxylation of cholesterol, the first and rate limiting step of bile acid synthesis. This is also the principal way to eliminate cholesterol. **CYP7B1** is primarily expressed in brain and catalyzes the synthesis of various neurosteroids and also the 7 α -hydroxylation of oxysterols.

CYP8A1 is the complementary enzyme to CYP5 in that it synthesizes prostacyclin in the arachidonic acid cascade. **CYP8B1** catalyzes the steroid 12- α hydroxylation in the cholic acid biosynthesis.

CYP11A1 is known as the mitochondrial side chain cleavage enzyme that converts cholesterol to pregnenolone, the first step in steroid hormone biosynthesis. Steroid hormone levels are under tight endocrine control via the P450 enzymes involved in their biosynthesis, which are transcriptionally regulated by ACTH (adrenocorticotrophic hormone) via intracellular cAMP. Genetic defects in CYP11A1 lead to a lack

of glucocorticoids, feminization and hypertension. **CYP11B1** is the mitochondrial 11-beta hydroxylase that synthesizes cortisol and corticosterone. Genetic defects in this gene lead to congenital adrenal hyperplasia. **CYP11B2**, aldosterone synthase, hydroxylates corticosterone at C-18. Genetic deficiency of CYP11B2 is the cause of congenital hypoaldosteronism.

CYP17 is the 17 alpha-hydroxylase and 17–20 lyase, two different reactions catalyzed by one enzyme and required for production of testosterone and estrogen, respectively. Defects in this enzyme affect development at puberty.

CYP19 is known as ►aromatase that synthesizes estrogen converting ring A of the steroid nucleus into an aromatic ring. Lack of this enzyme causes a lack of estrogen and failure of women to develop at puberty. Because estrogens are involved in breast cancer development, CYP19 is an important target to develop specific anti breast-cancer agents that inhibit the enzyme.

CYP21 catalyzes steroid C21 hydroxylation required for cortisol biosynthesis. Genetic defects in this gene cause congenital adrenal hyperplasia.

CYP24 is a 25-hydroxyvitamin D3 24-hydroxylase that degrades vitamin D metabolites.

CYP26 consists of three enzymes each representing a separate subfamily (Table 1) probably are all involved in retinoic acid hydroxylation. **CYP26A1** is an all trans retinoic acid hydroxylase which degrades retinoic acid, an important signalling molecule for vertebrate development. It acts through retinoic acid receptors. The other CYP26 isozymes are also retinoic acid hydroxylases.

CYP27A1 catalyzes the side chain oxidation (27-hydroxylation) in bile acid biosynthesis. Because bile acid synthesis is the only elimination pathway for cholesterol, mutations in the *CYP27A1* gene lead to abnormal deposition of cholesterol and cholestanol in various tissues. This sterol storage disorder is known as cerebrotendinous xanthomatosis. **CYP27B1** is the 1-alpha hydroxylase of vitamin D3 that converts it to the active vitamin form. The function of **CYP27C1** is not yet known.

CYP46 hydroxylates cholesterol at the 24-position, a reaction that appears to play a role for cholesterol homeostasis in the brain.

CYP51 catalyzes lanosterol 14-alpha demethylation required in the biosynthesis of cholesterol in mammalian liver but it is also expressed in sperm where it synthesizes meiosis-activating sterols. This enzyme is evolutionarily highly conserved in plants, fungi and animals, and bacteria and may be the ancestor of all eukaryotic P450s. CYP51 enzymes are important targets for cholesterol-lowering drugs, antifungal agents like ketoconazole, and herbicides.

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P-type ATPase(s)

Ion transporting ATPases that become autophosphorylated by the terminal phosphate group of ATP during the ion transporting process

►Na⁺/K⁺-ATPase

PACAP

►Pituitary Adenylate Cyclase-activating Polypeptide

PAF

►Platelet-activating Factor

PAG

►Periaqueductal Grey

Pain and Nociception

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Definition

Pain is a combination of sensory (discriminative) and affective (emotional) components. The sensory component of pain is defined as nociception.

Physiological pain constitutes a protective function as it warns the body against potentially damaging stimuli. Under certain circumstances, pain converts into a chronic disease and is manifest clinically as nociceptive hypersensitivity (►**hyperalgesia** and ►**allodynia**) or as spontaneous pain. Pathological pain syndromes include ►**neuropathic pain**, chronic inflammatory pain (e.g. rheumatic pain, Morbus Bechterew), neuralgias, ►**causalgias**, phantom limb pain, cancer pain and chronic ischemic pain (e.g. of cardiac origin), amongst many others.

Basic Mechanisms

Physiology of Pain and Analgesia

Table 1 illustrates the anatomical components of nociceptive system and their physiological functions. Harmful stimuli applied to the body activate the peripheral endings of primary sensory neurons, called ►**nociceptors**, whose cell bodies lie in the dorsal root ganglia (DRG) or the trigeminal ganglia. Distinct classes of nociceptors encode discrete intensities and modalities of pain. Receptor molecules that impart these specific properties to nociceptors constitute a novel area of intense investigation in pain research. These include the transient receptor potential channel V1 (TRPV1), which serves as a transducer of noxious thermal and chemical (protons) stimuli and the tetrodotoxin-resistant Sodium channels (Na_v1.7 and Na_v1.8), which act as potential generators or amplifiers in nociceptors [1].

Nociceptive information is conveyed from peripheral endorgans to the central nervous system by primary afferent fibres. Amongst these, the C-fibres and Aδ-fibres, which synapse with and activate numerous second order neurons located in the superficial laminae of the spinal dorsal horn, are of arch significance in context of the physiology as well as the pathophysiology of pain. The amino acid neurotransmitter, glutamate, serves as the primary nociceptive neurotransmitter at these synapses by activating several types of ►**glutamate receptors** such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, N-methyl-D-aspartate (NMDA) receptors and metabotropic glutamate receptors (mGluRs). Neurotransmission

at primary afferent-second order neuron synapses is further modulated and/or mediated by other agents that are released synaptically from primary afferent terminals upon peripheral nociceptive stimulation. These include neurotransmitters, such as ►**substance P** (Fig. 1), calcitonin gene related peptide, somatostatin and ATP and ►**neurotrophins** such as brain-derived neurotrophic factor (BDNF). It is believed that AMPA and kainate receptors mediate transmission of basal (physiological) pain whereas mGluRs and substance P-, NMDA-, calcium-permeable AMPA- and BDNF-receptors play a key role in induction and maintenance of pathological pain [2].

Afferent input from cutaneous and visceral nociceptors is known to converge on spinal neurons, which accounts for the referral of pain between visceral and cutaneous structures (e.g. cardiac pain gets referred to the chest and left upper arm in patients suffering from angina pectoris). Projection neurons in the spinal dorsal horn project to cell nuclei in supraspinal areas such as the thalamus, brainstem and midbrain. Of these, the synaptic junctions in the thalamus play a very important role in the integration and modulation of spinal nociceptive and non-nociceptive inputs. Nociceptive inputs are finally conducted to the cortex where the sensation of pain is perceived (Fig. 1). The mechanisms via which the cortex processes nociceptive inputs are only poorly understood.

Nociceptive activity can be modulated at several peripheral and central relay points in pain pathways. For example activation of myelinated primary fibres that conduct non-nociceptive input reduces the activity of spinal nociceptive projection neurons and thereby reduces the perception of pain. This is potentially one mechanism via which non-pharmacological approaches such as transelectrical nerve stimulation and acupuncture might relieve pain. Local interneurons exert excitatory or inhibitory influences on projection neurons in the spinal cord (Fig. 1) and thalamus. Furthermore, spinal nociceptive output is strongly modulated by descending systems that originate at supraspinal sites, such as the periaqueductal grey, rostroventromedulla and pons. Stimulation of these brain regions, either electrically or chemically, e.g. by morphine and other opiates, produces analgesia in humans. Both facilitatory as well as inhibitory descending influences on pain have been described [3]. Descending inhibitory pathways utilise monoamines such as noradrenaline and serotonin as neurotransmitters and terminate on nociceptive neurons in the spinal cord as well as on spinal inhibitory interneurons that store and release ►**opiods** (Fig. 1). The latter exert both pre-synaptic and post-synaptic inhibitory actions at primary afferent synapses in the spinal dorsal horn by activating specific opiod receptors. Thus, supraspinal pathways and local spinal circuits co-ordinately modulate incoming nociceptive

Pain and Nociception. Table 1 Physiology and pathophysiology of the nociceptive system

Anatomical component	Nociceptor terminal	Afferent	Spinal cord	Brain
Normal function	Transduction	Conduction	Processing, mono- and polysynaptic-reflexes	Processing, perception, emotional components of pain, polysynaptic reflexes
Pathological change	Sensitisation, desensitisation, changes in activation thresholds	Abnormal conduction, ectopic activity, sprouting	Hypersensitivity, hyposensitivity, changes in activation thresholds, expansion or reduction of receptive fields, deafferentation, spontaneous activity	
Etiology	Trauma, viral infections, ischemia, inflammation, genetic defects	Neuropathy, genetic defects	Peripheral inflammation, peripheral neuropathy, trauma, genetic defects, spinal cord injury, inflammation in the central nervous system	
Clinical manifestation	Primary hyperalgesia, allodynia, spontaneous pain		Secondary hyperalgesia, allodynia, spontaneous pain, aberrantly referred pain	
Pharmacological intervention	NSAIDs, local anesthetics, opioid analgesics, sodium-channel blockers		Opioid analgesics, NSAIDs, calcium-channel blockers	

signals [3]. Furthermore, the ►endocannabinoid system can strongly modulate pain via actions at almost all of the avenues in the somatosensory pain pathway described above.

Processes Underlying Pathological Pain

Common pathological alterations in nociceptive pathways and their underlying etiological factors are listed in Table 1. Damaging stimuli such as trauma, viral infections, noxious temperature or noxious pressure (e.g. by tumour outgrowth) can elicit both short-term and long-term changes in the activity of nociceptors and/or central nociceptive neurons. Peripheral effects include the release of chemoactive substances from damaged cells, blood vessels or from nociceptors themselves (Fig. 1), which either activate or sensitise nociceptors. These peripheral mechanisms are typically short-term in nature and are predominantly manifest as primary hyperalgesia. Chronic nociceptor activity can also produce long-lasting sensitisation of nociceptors by transcriptional regulation of genes encoding important nociceptive molecules in dorsal root or trigeminal ganglia neurons, thereby leading to the up- or down-regulation of receptors, enzymes or other signalling molecules on primary afferent terminals [1, 2]. Such molecular events lead to alterations in transduction as well as conduction properties of primary afferents.

Nociceptive neurons in the spinal cord as well as in higher centres such as the thalamus and cortex can also undergo alterations in activity following chronic peripheral changes and trauma (Table 1). These changes are typically long-term in nature and lead to the clinical syndromes of centrally maintained pain (secondary hyperalgesia, allodynia, spontaneous pain). Alterations

in the synaptic efficacy of primary afferent-first order neuron synapses constitute a prime underlying mechanism [2]. At the molecular level, these are brought about by long-term changes in the expression and signalling mechanisms of receptors for nociceptive neurotransmitters and neuromodulators on pre-synaptic terminals and in post-synaptic cells in the spinal cord [2]. Recent studies suggest that spinal microglia play an important role in mechanisms underlying neuropathic pain by secreting pro-nociceptive neuromodulators such as ATP, BDNF etc.

Pharmacological Intervention

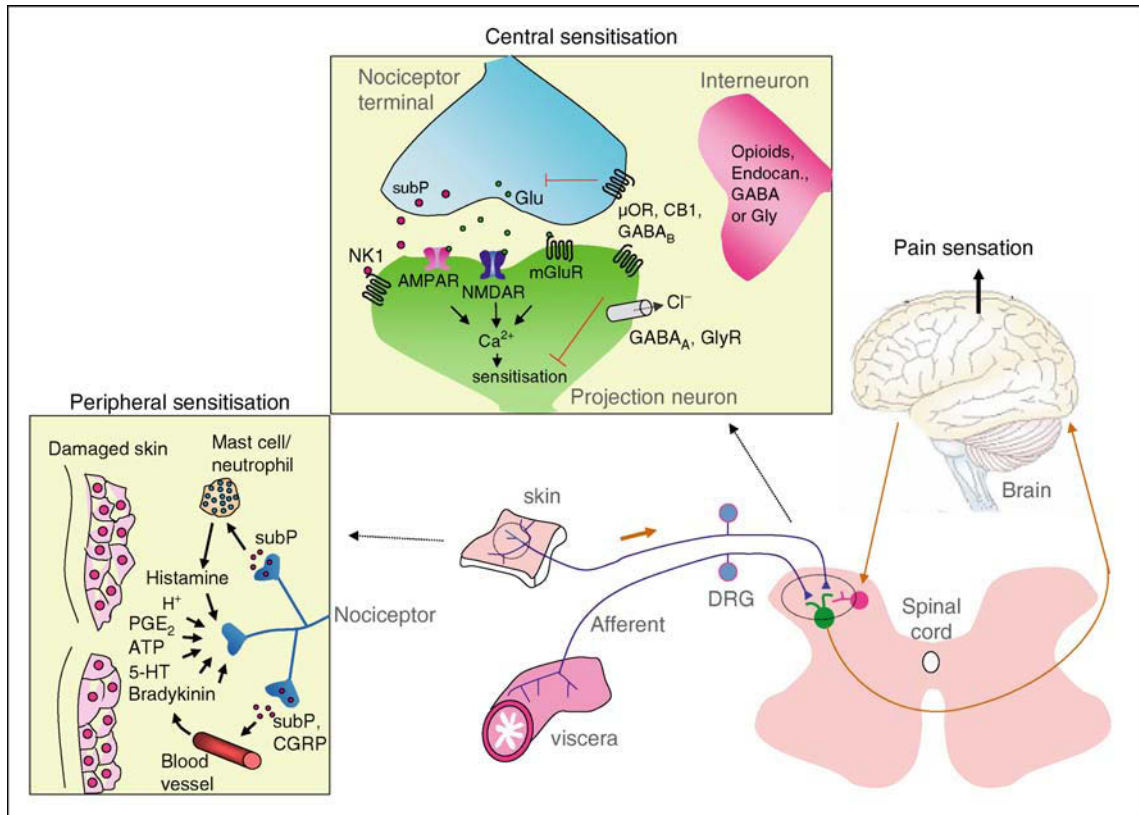
Avenues Targeted by Contemporary Analgesics

Synthesis Pathways for Peripheral Chemoactive Agents

Prime examples for this class of analgesics are non-steroidal anti-inflammatory drugs (►NSAIDs, e.g. aspirin, ibuprofen), which act by blocking cyclooxygenases (COX-1 and/or COX-2), the enzymes responsible for synthesis of prostaglandins, mainly prostaglandin E₂ (PGE₂), in peripheral tissues and thereby preventing or terminating the sensitisation of nociceptors (Fig. 1). A central component to NSAID-produced analgesia has been described, the mechanisms underlying it being complex. NSAIDs are particularly effective against pain in the extremities, headache, migraine and dental pain, and are generally insufficient in treating chronic central pain.

Nociceptive Conduction by Primary Afferents

Anaesthetics such as lidocaine and tetracaine reversibly block the generation and conduction of action potentials in primary afferent fibres without inducing systemic effects when applied locally to dermatotomes. Their



Pain and Nociception. Figure 1 Schematic representation of nociceptive mechanisms and their sensitisation: Noxious stimuli such as protons (H^+), heat etc., which are applied to end organs, activate nociceptors (magnified in inset on the left). Injury leads to the release of prostaglandins such as prostaglandin E_2 (PGE_2), serotonin (5-HT), nerve growth factor (NGF) and ATP from damaged cells, bradykinin (BK) from blood vessels and substance P (subP) from nociceptors. These agents either activate nociceptors directly or sensitise them to subsequent stimuli by parallel activation of intracellular kinases by G-protein-coupled receptors and tyrosine kinase receptors. Primary nociceptive afferents (C-fibres, A δ -fibres) of dorsal root ganglion (DRG) neurons synapse on second order neurons in the spinal dorsal horn (magnified in upper inset). Here, glutamate (Glu) and subP released from nociceptor terminals activate glutamate receptors (NMDAR, AMPAR, mGluRs) and neurokinin-1 receptors (NK-1), respectively, located post-synaptically on spinal neurons and induce sensitisation by modulating calcium (Ca^{2+}) signalling. These synapses are negatively modulated by spinal inhibitory interneurons, which release endogenous opioids (activate μ OR), endocannabinoids (Endocann.; activate CB1), glycine (Gly; activates GlyR) or γ -amino-butyric acid (GABA; activates GABA $_A$ and GABA $_B$). Spinal projection neurons convey nociceptive information to the brain and brainstem. Activation of descending noradrenergic and/or serotonergic systems, which originate in the brain and brainstem, leads to the activation of spinal inhibitory interneurons thereby resulting in anti-nociception.

main action is to block voltage-dependent sodium channels by physically plugging the transmembrane pore. The degree of blockade is inversely proportional to the extent of myelination on the nerve fibres. Therefore, the nociceptive C-fibres and A δ -fibres are blocked preferentially in comparison with thickly myelinated non-nociceptive primary afferents.

Opioid Receptors

Similar to endogenous opioids, opiates like morphine and other synthetic opioids activate G-protein-coupled receptors which couple to G-proteins of the $G_{i/o}$ family.

They act as analgesics by inhibiting release of nociceptive neurotransmitters from primary afferent terminals as well as by depressing post-synaptic potentials on second order neurons. Opioid receptors are also present on some nociceptors and their expression and peripheral transport is increased upon peripheral inflammation. Peripheral opioid analgesia has been established in animal models. Although clinical studies have yielded mixed results so far, this field holds great promise. Despite side effects, such as euphoria, dysphoria, sedation, respiratory depression and obstipation and tolerance and dependence phenomena which arise upon

chronic use, opioid analgesics currently constitute prime therapeutics used in the treatment of labour pain and severe chronic pain, particularly that arising from inflammation, cancer or central mechanisms.

Blockers of Sodium and Calcium Channels

Anticonvulsants, which are blockers of ion-channels, have been empirically found to be effective in symptomatic management of neuropathic pain, probably owing to the similarities between the pathophysiological phenomena underlying epilepsy and neuropathic pain [4]. Recent clinical trials support the use of sodium channel blockers, e.g. carbamazepine, lamotrigine and calcium channel blockers, e.g. Gabapentin and Ziconitide in the treatment of neuropathic pain syndromes such as painful diabetic mononeuropathy, post-herpetic neuralgia, trigeminal neuralgia and central pain [4].

Antidepressives

Antidepressive agents which increase synaptic availability of serotonin and noradrenaline have been empirically found to be efficacious against chronic pain, particularly that of neuropathic origin.

Novel and Emerging Drug Targets

Glutamate Receptors

The NMDA subtype of glutamate receptors are key mediators of chronic nociceptive phenomena in the spinal cord and the thalamus. NMDA receptor antagonists like MK-801, d, 1–2-amino-5-phosphonvaleric acid etc. effectively inhibit several kinds of chronic pain, such as neuropathic and inflammatory pain, in corresponding animal models [5]. In clinical trials, NMDA receptor antagonists were found to effectively inhibit chronic pain in humans but also to produce severe side effects on the central nervous system, including psychotomimetic effects, which limit their therapeutic application. Clinically used agents which block NMDA receptors weakly include ketamine and dextromethorphan. Development of novel drugs which selectively block specific-subtypes of NMDA receptors, e.g. NR2B-containing NMDA receptors and calcium-permeable AMPA receptors offer new hope for achieving specific analgesic effects. Furthermore, drugs acting on specific mGluR subtypes involved in nociception are currently being developed as potential analgesics.

GABA, Glycine and Cannabinoid Receptors

GABA is the most prominent inhibitory neurotransmitter in the mammalian nervous system and acts via ►GABA receptors. Activation of GABA_B receptors by GABA released from local spinal interneurons (Fig. 1) negatively modulates nociceptive transmission in the spinal cord. Agonists at GABA_B receptors

(e.g. baclofen) inhibit pain in animal models and in humans, but also produce muscle relaxation. There is scope for finding drugs selectively activating GABA_B receptors, GABA_A receptors and glycine receptors expressed in nociceptive neurons or in the spinal dorsal horn in order to block pain without affecting motor function. Furthermore, Gi-coupled receptors for cannabinoids (CB₁ and CB₂) constitute the most promising current targets for developing novel and effective analgesics.

Nociceptive Transducers

These molecules largely include membrane-bound cation channels which serve as receptors for heat (TRPV1, TRPV2), cold (ANKTM1) and protons (TRPV1, acid-sensing ion channels) etc. Several splice variants of acid-sensing ion channels as well as tetrodotoxin-resistant sodium channels (Na_v1.8, Na_v1.7) are selectively expressed in nociceptors [4]. Pharmacological intervention at these channels therefore holds promise for producing analgesics with fewer side effects on other organs. It is likely, however, that these agents will also block acute, physiological pain.

Neurotrophin Receptors

Neurotrophins such as nerve growth factor (►NGF) and ►BDNF are now emerging as important modulators of nociceptor activity as well as central mechanisms of pain. Receptors and signalling mechanisms involved in neurotrophin-produced modulation of nociception are areas of intensive current research and represent novel avenues for pharmacological intervention in chronic pain states, e.g. via humanised monoclonal antibodies against NGF. Furthermore, because of their trophic effects on injured nerves, targeting neurotrophins may be useful in regeneration of traumatised nerves as well as in arresting and reversing disease processes underlying sprouting of nociceptors in diseased states.

- Analgesics
- Kinins
- Non-steroidal Anti-inflammatory Drugs
- Opioid Systems

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Pain Medication

► Analgesics

Palmitoylation

Palmitoylation is the post-translational lipid modification of cysteine-residues in a variety of proteins.

► Lipid Modifications

PAMPs

Pathogen-associated molecular patterns (PAMPs) are microbial components derived from pathogens such as bacteria, viruses, fungi, and parasites. PAMPs are specifically recognized by the Toll-like receptors (TLRs), which are expressed by cells of the innate immune defense such as dendritic cells and macrophages. PAMPs comprise for very diverse components including lipopolysaccharide (LPS) from Gram-negative bacteria, lipoproteins and lipopeptides, flagellin, bacterial and viral unmethylated CpG DNA, or double-stranded RNA (dsRNA) produced by most replicating viruses.

- Nuclear Factor Kappa B
- Toll-like Receptors

Pancreatic β -cell

The predominant cell type in the pancreatic islets of Langerhans. The main secretory product of the β -cell is the peptide hormone insulin which has vital actions for the control of nutrient homeostasis and cellular differentiation.

- Diabetes Mellitus
- Oral Antidiabetic Drugs
- Ca^{2+} Channel Blockers

Pancreatic Polypeptide

The hormone pancreatic polypeptide (PP) is a 36 amino acid peptide, which is closely related to *neuropeptide Y* and *peptide YY*. PP is mainly found in pancreatic cells distinct from those storing insulin, glucagon or somatostatin. It acts on receptors that belong to the family of *neuropeptide Y* receptors, particularly on the Y_4 subtype.

► Neuropeptide Y

Pantothenic Acid

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Synonyms

(R)-(+)-N-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)- β -alanine)

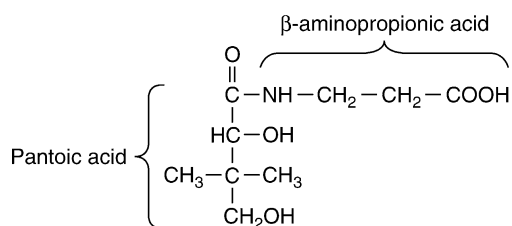
Definition

Pantothenic acid is an unstable, highly hygroscopic viscous water and alcohol-soluble oil of light yellow color. The vitamin is stable to heat and light. In pharmaceutical preparations, Na^+ - or Ca^{2+} -salts of the alcohol panthenol are more commonly used because it has an overall higher stability. Pantothenic acid occurs in most food stuffs. Liver, kidney, and brain contain particularly high concentrations of pantothenic acid, yet numerous small contributions from other dietary sources, e.g., fruits, vegetables, milk and milk products, cereals or pulses, are more important for the coverage of daily requirements [1,2].

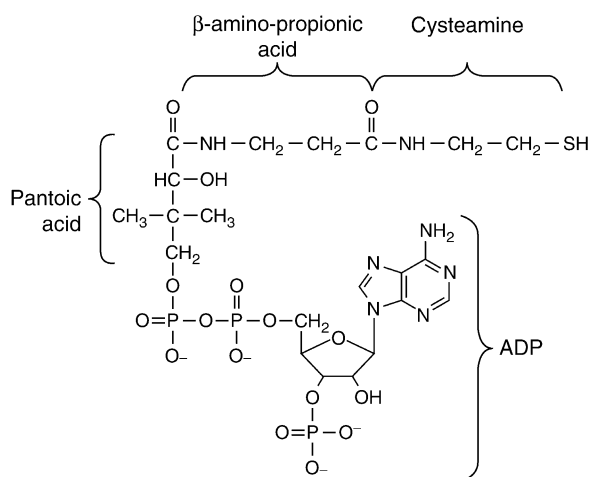
Figure 1 shows the structure of pantothenic acid ((R)-(+)-N-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)- β -alanine). Only D(+)-pantothenic acid occurs naturally and is biologically active. The alcohol (R)-panthenol (= (D)-panthenol) shows biological activity as well.

Mechanism of Action

Pantothenic acid is an essential component of coenzyme A (CoA) (Fig. 2) and – as pantetheine – of fatty acid synthase. The HS-group of cysteamine is



Pantothenic Acid. Figure 1 Structure of pantothenic acid.



Pantothenic Acid. Figure 2 Structure of co-enzyme A.

in both cases the active site for the binding of acyl- or acetyl-residues. There is also a pantothenate-dependent step in the synthesis of leucine, arginine, and methionine.

The main role of CoA is acyl- and acetyl-group transfer and condensation. CoA plays a vital role in the metabolism of carbohydrates, fatty acids, and nitrogen compounds. In addition to the role in energy generation and molecular syntheses, pantothenic acid participates in regulating numerous proteins by donating acetyl- and fatty acyl-modifying groups, which alter the location and/or activity of the acylated protein [2].

Clinical Use (Including Side Effects)

Panthenol is frequently used in ointments and solutions for the treatment of burns, anal fissures, and inflammation of the conjunctiva. The vitamin has to be substituted in patients on total parenteral nutrition and in those who regularly undergo dialysis. Hypervitaminosis has not been observed for doses up to 5 g/d (22). Furthermore, the administration of pantothenic acid leads to improved surgical wound healing due to its antiinflammatory properties.

- Folic Acid
- Niacin
- Biotin
- Vitamin B1
- Vitamin B2
- Vitamin B6
- Vitamin B12
- Vitamin C

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Parasite

Eukaryotic pathogen, except fungi.

- Antiprotozoal Drugs

Parasympathetic Nervous System

The autonomic nervous system, which regulates functions that occur without conscious control, consists of two major divisions, the sympathetic and the parasympathetic nervous system. In the periphery, it consists of nerves, ganglia, and plexuses that provide innervation to the heart, blood vessels, glands, other visceral organs, and smooth muscle in various tissues. The parasympathetic nervous system is concerned primarily with conservation of energy and maintenance of organ function during periods of minimal activity. Acetylcholine is the neurotransmitter at all preganglionic autonomic fibers and at all postganglionic parasympathetic fibers.

- Muscarinic Receptors

Parasympatholytics

- Muscarinic Receptors

Parasympathomimetics

► Muscarinic Receptors

Parathyroid Hormone

A major regulator of bone metabolism and calcium homeostasis, parathyroid hormone (PTH) is stimulated through a decrease in plasma ionised calcium and increases plasma calcium by activating osteoclasts. PTH also increases renal tubular calcium re-absorption as well as intestinal calcium absorption. Synthetic PTH (1–34) has been successfully used for the treatment of osteoporosis, where it leads to substantial increases in bone density and a 60–70% reduction in vertebral fractures.

- Bone Metabolism
- Ca^{2+} -Sensing Receptor

Paraventricular Nucleus

The paraventricular nucleus in the hypothalamus is located adjacent to the third ventricle and has been identified as a satiety center. Neurons in the paraventricular nucleus produce neuropeptides which inhibit feeding when injected into the brain (thyrotropin-releasing hormone (TRH), corticotropin-releasing hormone (CRH), oxytocin).

- Appetite Control

Parkin

Parkin is a ubiquitin ligase encoded by a gene affected in autosomal recessive juvenile parkinsonism (AR-JP). This gene is located on chromosome 6 and encodes a protein of 465 amino acid residues with moderate similarity to ubiquitin at the amino terminus and a RING-finger motif at the carboxy terminus.

- Ubiquitin/Proteasome System

Parkinson's Disease

Parkinson's disease (PD) is a progressive disease characterized by akinesia, muscle tremor, and rigidity resulting from the degeneration of melanin-containing cells of the substantia nigra and the resulting reduction in brain dopamine levels. PD initially responds to L-DOPA therapy but the disease eventually enters a refractory phase possibly due to the continued loss of cells. Drugs that destroy dopaminergic neurons, block dopamine synthesis or antagonize D2-like receptors have PD-like effects.

- Dopamine System
- Anti-Parkinson Drugs
- Neurotrophic Factors
- Monoamine Oxidase and their Inhibitors
- Ubiquitin/Proteasome System
- Catechol-O-Methyl Transferase and its Inhibitors

Partial Agonists

Agonists that in a given tissue, under specific conditions, cannot elicit as optimal an effect (even when applied at high concentration, so that all the receptors should be occupied) as can another agonist acting through the same receptors in the same tissue. Partial agonists have partial efficacy at a receptor relative to a full agonist.

- Drug-Receptor Interaction
- Nuclear Receptors

Parvalbumin

Parvalbumin is a cytosolic protein expressed mainly in skeletal muscles and brain.

- Annexins
- Ca^{2+} -Binding Proteins

PAS Domain

PAS domains are protein domains, encompassing about 250–300 amino acids, which in higher eukaryotes function as surfaces for both homotypic interactions

with other PAS proteins and heterotypic interactions with cellular chaperones, such as the 90-kD heat shock protein (Hsp90). They are named according to the first letter of each of the three founding members of the family of PAS domain containing proteins, PER, ARNT and SIM. Most of the PAS proteins also contain basic-helix-loop-helix (bHLH) motifs immediately N-terminal to their PAS domain. The HLH domains participate in homotypic dimerization between two bHLH-PAS proteins, and they position the basic region to allow specific contacts with the major groove of target regulatory elements found in DNA. Important members of the PAS superfamily are the aryl hydrocarbon receptor (AHR), which together with ARNT forms the receptor for a class of co-planar polyhalogenated aromatic hydrocarbons like dioxins. Although structurally distinct from the group of nuclear receptors, the Ah receptor functions in similar fashion. In the absence of ligand, the receptor resides in the cytosol as an inactive complex with Hsp90. Binding of dioxins enables the receptor to translocate to the cell nucleus and to bind to specific DNA recognition motifs. Other members of the PAS superfamily include the products of the period and clock genes, which are involved in the regulation of circadian rhythms as well as hypoxia-inducible factor (HIF1), which mediates the effect of hypoxia on the regulation of the expression of a variety of genes, including those encoding erythropoietin or vascular endothelial growth factor.

Passive Diffusion

The passage of a small and/or highly lipophilic molecule through the membrane phospholipid bilayer according to the gradient of its concentrations across the plasma membrane. It is slower than facilitated diffusion, which, however, also follows the gradient of solute concentrations across the membrane.

Patch-Clamp Method

The patch-clamp technique is based on the formation of a high resistance seal (10^9 – $10^{10}\Omega$) between the tip of a glass micropipette and the cell membrane it touches (gigaohm-seal). This technique allows recordings of ionic currents through single ion channels in the intact cell membrane and in isolated membrane patches at a

defined membrane potential (voltage-clamp). Variations of the technique include whole-cell voltage-clamp recording and recordings in inside-out or outside-out membrane configurations.

PC: Proconvertase

► Incretin Hormones

PCI

► Percutaneous Coronary Intervention

PDE

► Phosphodiesterases

PDGF

► Platelet-derived Growth Factor

PDZ Domain

Synonyms

Post synaptic density protein/Drosophila disc large tumor suppressor/zonula occludens-1 protein

Definition

Protein-protein interaction domain that binds to short peptide motifs at the C-terminal of target proteins. Particularly important in spatial organization of receptors and ion channels.

► Adaptor Proteins

PEG

Polyethylene glycol (PEG) is a polymer. PEG refers to an oligomer or polymer of ethylene oxide. PEG is a liquid or low-melting solid, depending on its molecular weight. Derivatives of PEG are in common use, the most common derivative is the methyl ether (methoxypoly(ethylene glycol)), abbreviated mPEG. PEGylation, the process by which polyethylene glycol chains are attached to proteins or peptide drugs, making these more resistant to degradation by proteolytic enzymes. By increasing the molecular mass of proteins/peptides and shielding them from proteolytic enzymes, PEGylation improves pharmacokinetics.

► Tumor Necrosis Factor (TNF)

► Cadherins/Catenins
► Glucocorticoides
► Immunosuppressive Agents

Pellagra

The clinically manifest form of niacin deficiency is called pellagra. Early symptoms are unspecific, including sleep disturbance, lack of appetite, weight reduction, diarrhea, and abdominal pain. Typical symptoms of more severe stages relate to skin (symmetrical, pigmented, and itching areas on sun-exposed skin), intestinal tract (glossitis, stomatitis, vomiting), and nervous system (pain and numbness of extremities, peripheral neuritis, psychological changes).

► Niacin

Pemphigus Blistering Disease

Pemphigus describes a group of rare autoimmune blistering diseases of the skin caused by autoantibodies to desmosomal components. These antibodies lead to a loss of adhesion between keratinocytes, called acantholysis. In pemphigus vulgaris, which is caused by autoantibodies to desmoglein 3, the blisters are located in the suprabasal layer, whereas in pemphigus foliaceus, which is caused by autoantibodies to desmoglein 1, the blisters occur within the upper layers of the epidermis. The major therapeutic strategy in pemphigus is chronic immunosuppressive therapy with glucocorticosteroids in combination with immunosuppressive adjuvants.

Penicillin

► β -Lactam Antibiotics

Penicillin Binding Protein

Synonyms

PBP

Definition

Proteins identified by their ability to bind labelled β -lactam antibiotics in vivo and in vitro. The intrinsic activities of PBPs include transglycosylase/transpeptidase, carboxypeptidase and endopeptidase activities required for the formation of the bacterial murein sacculus forming the bacterial cell wall. The enzymes are located in the cytoplasmic membrane.

► Bacterial Resistance to Antibiotics

Pentasaccharide

Heparin.

► Anticoagulants

Peptide Mass Fingerprint

Peptide mass fingerprinting (PMF) is a mass spectrometry based method for protein identification. The protein is cleaved by an enzyme with high specificity (trypsin, Lys-C, Asp-N, etc.) or chemical (CNBr). The peptide mixture generated is analyzed by matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI).

mass spectrometry. The determined set of masses (mass fingerprint) is characteristic for the protein present and is used to search peptide masses generated by theoretical fragmentation of protein sequences in databases.

► Proteomics

Peptide YY

The hormone peptide YY (PYY) is a 36 amino acid peptide, which is closely related to *neuropeptide Y* and *pancreatic polypeptide*. PYY is predominantly synthesised and released by intestinal endocrine cells, and can also coexist with glucagon in pancreatic acini and enteroglucagon in endocrine cells of the lower bowel. It acts on the same receptors as *neuropeptide Y*. The endogenous long C-terminal PYY fragment PYY_{3–36} is a biologically active and subtype-selective metabolite.

► Neuropeptide Y

Peptidoglycans

Peptidoglycans are covalently closed net-like polymers forming the rigid matrix of the bacterial cell wall. Glycan chains of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues are substituted for by L-alanyl-γ-D-glutamyl-L-diaminoacyl-D-alanine stem tetrapeptides, and cross-linked through direct interpeptide linkages or cross bridges. Bacteria that are actively multiplying (i.e. in the exponential phase of growth) manufacture a (4–3) peptidoglycan in a penicillin-susceptible manner. Cross linking involves the carbonyl of the D-alanine at position 4 of a stem peptide and the ω-amino group of the diaminoacid residue at position 3 of another stem peptide. Bacteria also manufacture a (3–3) peptidoglycan in a penicillin-resistant manner, conferring intrinsic resistance to β-lactam antibiotics. Cross linking involves the carbonyl of the diaminoacid residue at position 3 of a stem peptide and the ω-amino group of the diaminoacid residue also at position 3 of another stem peptide.

- β-Lactam Antibiotics
- Microbial Resistance to Drugs
- Quinolones
- Ribosomal Protein Synthesis Inhibitors

Peptidyl Transferase Center

The peptidyl transferase center is the site of peptide bond formation on the ribosome and the target for a chemically diverse set of antibiotics. It is located in a cavity on the 50S subunit that leads into a peptide exit tunnel that passes through the body of the 50S subunit. The active site is in a deep cleft that is packed with nucleotides from the highly conserved internal loop in domain V of 23S ribosomal RNA (rRNA), termed the peptidyl transferase loop. In general, the single-stranded nucleotides in the loop are the closest to the active site, with the helices around the internal loop radiating away from the catalytic center. The peptidyl transferase loop has previously been implicated as a component of the catalytic site through the localization of chemical footprints and crosslinks from both antibiotics and transfer RNAs (tRNAs) to this region. In addition, for many of the peptidyl transferase antibiotics, mutations at single nucleotides in the peptidyl transferase loop produce antibiotic resistance.

► Ribosomal Protein Synthesis Inhibitors

Peptidyl-Dipeptidase

An exopeptidase that sequentially releases dipeptides from the C-terminus of a protein or peptide. An example is angiotensin-converting enzyme (also known as peptidyl-dipeptidase A; *MEROPS* XM02-001), which plays an important role in the control of blood pressure by converting angiotensin I to angiotensin II. Peptidyl-dipeptidases are included in Enzyme Nomenclature sub-subclass 3.4.15.

► Non-viral Peptidases

PER1

(Period 1) Clock gene and transcriptional repressor and negative limb (complex with CRY, PER2) of molecular circadian oscillators, essential for circadian clock plasticity and entrainment.

► Orexins

Percutaneous Coronary Intervention

Percutaneous coronary intervention (PCI) is one of a host of techniques performed by using a catheter inserted via a major limb artery that aims to relieve narrowing of coronary arteries. For example, percutaneous transluminal coronary angioplasty (PTCA) is the classic PCI that uses a catheter-directed balloon to dilate a stenotic coronary artery, and more recent PCIs include stent implantation, rotational atherectomy, and laser angioplasty.

► Anticoagulant

Periaqueductal Grey

The periaqueductal grey (PAG) is a major part of the descending inhibitory antinociceptive system. The periaqueductal grey area is localized in the midbrain, a small area of grey matter surrounding the central canal. Neurons of the PAG are excited by opioids leading to the stimulation of cells in the nucleus raphe magnus, which, via the dorsolateral funiculus, send projections to the dorsal horn which are mainly serotonergic. These serotonergic and opioidergic neurons lead to the inhibition of nociceptive transmission in the dorsal horn.

► Nociception

Pericytes

Pericytes are mural cells which stabilise capillaries and control functions of capillary endothelial cell properties.

► Angiogenesis and Vascular Morphogenesis

Peripheral Nerve

A peripheral nerve comprises different axons responsible for different modalities. It may contain efferent myelinated motor fibres, efferent unmyelinated autonomic fibres

or afferent myelinated and unmyelinated sensory fibres. Depending on size, myelination and conduction velocity fibres are named A-, B- or C-fibres.

► Local Anaesthetics

Peripheral Neuropathy

Peripheral neuropathy is degeneration of peripheral nerves. Because motor and sensory axons run in the same nerves, usually both motor and sensory functions are affected in this disease. Neuropathies may be either acute (e.g., Charcot-Marie-Tooth disease) or chronic (e.g., Guillain-Barre syndrome) and are categorized as demyelinating or axonal.

► Neurotrophic Factors

Peroxisome Proliferator-Activated Receptors

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Synonyms

PPARs; PPAR α : NR1C1; PPAR δ : NR1C2, PPAR β , NUC1, FAAR (fatty acid-activated receptor); PPAR γ : NR1C3

Definition

The peroxisome proliferator-activated receptors (PPARs), members of the ►nuclear receptor superfamily, are ►transcription factors and their activity is modulated by the binding of ligand. Ligand binding (►drug-receptor interaction) elicits (i) binding of the receptor to specific DNA response elements located in close proximity to the promoter region of target genes and (ii) activation of target gene transcription. Three distinct PPAR isotypes exist: PPAR α , PPAR γ , and PPAR δ . The PPARs play essential roles in mediating lipid and glucose metabolism and homeostasis. In addition, recent evidence suggests that PPARs have important functions in additional diverse physiological processes

including ►inflammation and ►atherosclerosis, reproduction and fertility, ►Cell Cycle Control, skin biology and wound healing, cancer, and normal development of the nervous system.

Basic Characteristics

The Nuclear Receptor Family

The nuclear receptor (NR) family of transcription factors is quite large (65 distinct NR genes identified throughout the animal kingdom from nematodes to man) and may be categorized into three sub-groups: classic hormone receptors such as the glucocorticoid, estrogen, thyroid, retinoic acid, and vitamin D receptors; “sensor” receptors such as the PPARs, the liver X receptor (LXR), the farnesol X receptor (FXR), and the retinoid X receptor (RXR); and ►orphan nuclear receptors such as apolipoprotein A-I regulatory protein-1 (ARP-1) and chicken ovalbumin upstream promoter transcription factor (COUP-TF). In general, these categories describe characteristics of ligand binding. Ligands specific for the orphan receptors, if any exist at all, have not been identified to date. Classic hormone receptors bind specific ligands with high affinity. On the other hand, a broad range of lipophilic molecules bind to the “sensor” receptors, generally with lower affinity as compared to hormones that bind to the classical hormone receptors. The molecules that bind and activate the PPARs are most likely fatty acid-derived key metabolic substrates and intermediates that, by binding and modulating receptor activity and target gene transcription, provide a mechanism for rapid response to changes in metabolic status. The natural (endogenous) and synthetic (exogenous) ligands of the PPAR isotypes will be described in greater detail below. The current opinion is that alterations in the levels of fatty acids or fatty acid-derived molecules, resulting from changes in food availability (fasting/feeding/exercise) or pathophysiological conditions (chronic inflammation, ►insulin resistance, type 2 ►diabetes mellitus, atherosclerosis, or cancer), signal the appropriate PPAR isotypes to mediate lipid catabolism or storage.

The PPAR Isotypes

All members of the NR family are thought to have evolved from a common ancestral orphan receptor. Phylogenetic analysis suggests the ancestral NR appeared early during evolution, and the large number of family members has resulted from two distinct waves of gene duplication. The first wave generated an ancestral PPAR, whereas the vertebrate-specific second wave gave rise to the distinct PPAR isotypes. Once the three isotypes emerged, each is thought to have independently developed the ability to bind various ligands. Each isotype is encoded by a distinct gene. The first PPAR gene identified was mouse PPAR α . Initially

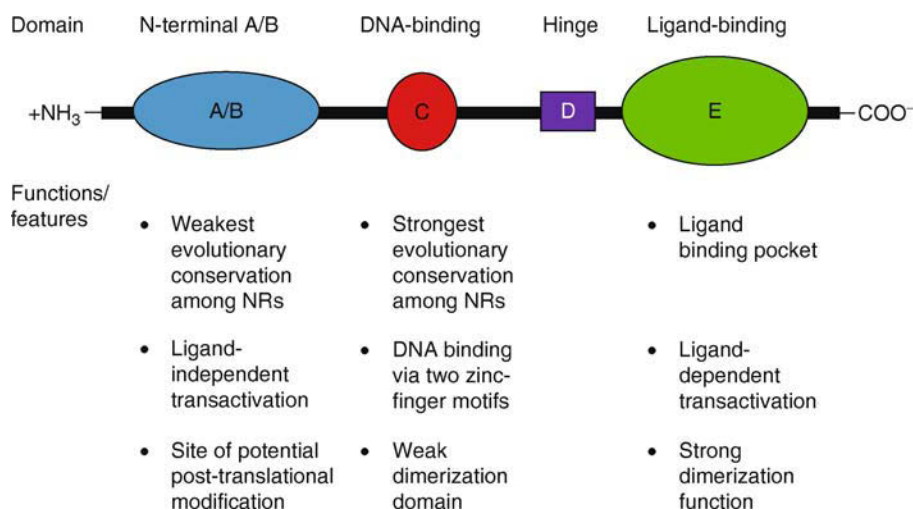
classified as an orphan receptor, the mouse PPAR α was subsequently found to be activated by peroxisome proliferators (PPs), hence the name peroxisome proliferator-activated receptor. Peroxisome proliferators are a structurally diverse group of compounds that induce proliferation of peroxisomes and hepatocarcinogenesis in rodents and include the widely used hypolipidemic fibrate drugs, such as fenofibrate (Tricor[®]) and gemfibrozil (Lopid[®]), as well as phthalate ester plasticizers, some herbicides, and chlorinated hydrocarbons. Peroxisomes are small membrane-bound compartments present in the cells of most eukaryotes and contain the enzymes for β -oxidation of long-chain fatty acids. Treatment of rodents with PPs predominantly affects the liver, with increased liver size due to both cellular hypertrophy and hyperplasia. In addition to increases in the size and number of peroxisomes, transcription of the genes encoding enzymes responsible for peroxisomal β -oxidation is also induced. Peroxisome proliferation and hepatocarcinogenesis are not seen in humans.

Modular Domain Structure of PPARs

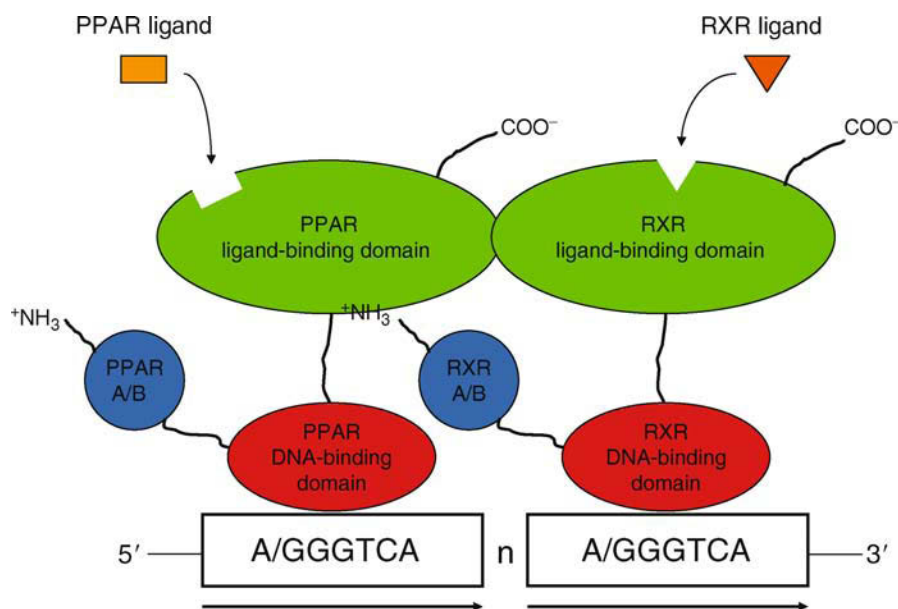
Consistent with other members of the NR family, the PPARs have a modular structure composed of five distinct regions (A–E) as shown in Fig. 1. The N-terminal A/B region is the least conserved among the NRs, and its length differs significantly among the NRs. Furthermore, this region is often a site for post-translational modification of receptor activity. The A/B region contains a weak ligand-independent transcriptional activation function (AF-1). Region C, the most highly conserved region among the NRs, contains the DNA-binding domain with its two zinc-finger motifs, the hallmark characteristic of the NR family. The D region serves as a hinge, allowing the more conserved and structured C and E domains to swivel to accommodate multiple conformations. Region E is also highly conserved and contains several distinct functions: the ligand-binding domain, the ligand-dependent transcriptional activation function (AF-2), and the primary dimerization domain.

DNA Recognition by PPARs

Response elements bound by members of the NR family consist of a consensus DNA hexamer (A/GGGTCA) arranged as either a single half-site or, more often, as two tandem half-sites arranged as direct, inverted (palindrome), or everted repeats. The spacing between the half-sites also plays a role in determining specificity for different NRs. In general, the majority of PPAR responsive elements (PPAR-RE) identified in genes responsive to PPAR ligands have a DR1 (direct repeat separated by 1 spacer nucleotide) structure, though other more complex PPAR-REs have been reported. As shown in Fig. 2, PPARs only bind DNA



Peroxisome Proliferator-Activated Receptors. Figure 1 Common structural and functional features of nuclear receptor transcription factors. Consistent with other members of the nuclear receptor superfamily, the PPARs have a modular domain structure consisting of domains A/B, C, D, and E. Each domain is associated with specific functions.

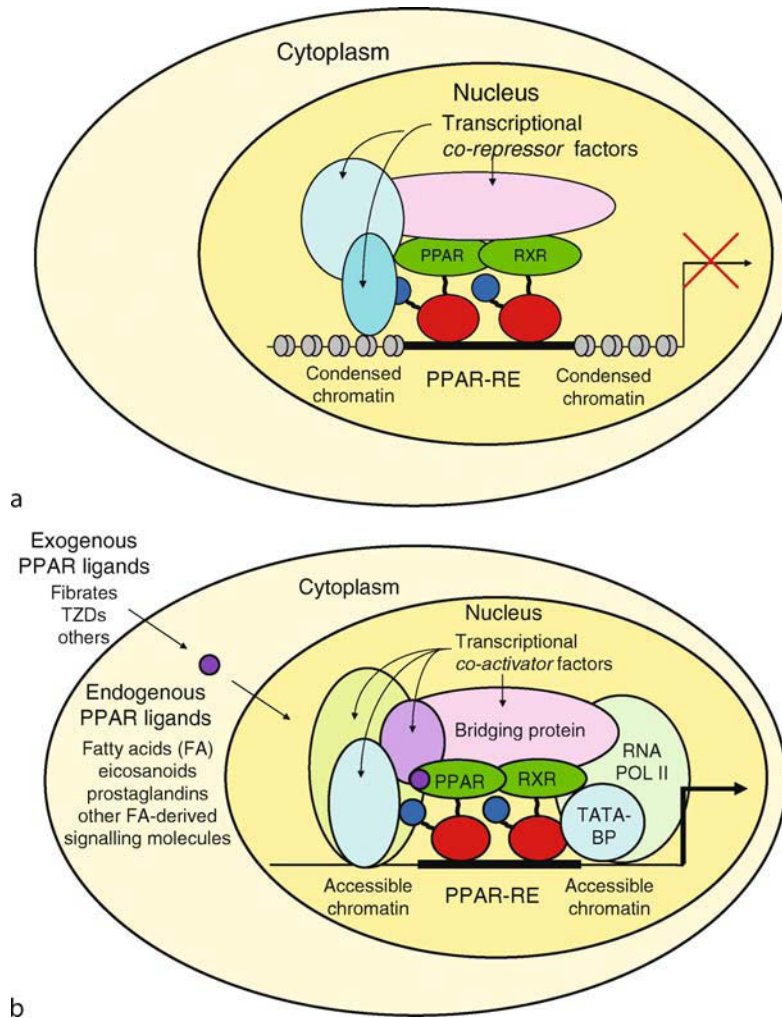


Peroxisome Proliferator-Activated Receptors. Figure 2 Binding of PPAR:RXR heterodimers to DR1 PPRE-responsive elements. *Abbreviations:* DR1, a direct repeat organization of the A/GGGTCA hexamer half-site separated by a single nucleotide spacer.

as heterodimers with the ►retinoid X receptor (RXR), another “sensor receptor” member of the NR family, and cannot bind DNA target elements as homodimers or monomers. There is a distinct “polarity” of the PPAR:RXR heterodimer, with the PPAR occupying the 5′ position and RXR the 3′ position when bound to the DR1 PPAR-RE.

Mechanism of Transcriptional Activation by PPAR Ligands

Figure 3 provides a very general overview of transcriptional activation in response to a PPAR ligand. Fig. 3a shows the schematic representation of a PPAR target gene in the absence of PPAR ligand. Co-repressor proteins bound to both unliganded PPAR and RXR



Peroxisome Proliferator-Activated Receptors. Figure 3 Transcription of PPAR target genes. A schematic representation of the transcription of PPAR-regulated genes in the absence (a) and presence (b) of PPAR ligand. *Abbreviations:* PPAR-RE, peroxisome proliferator-activated receptor-response element; RNA Pol II, RNA polymerase II; TATA-BP, TATA-binding protein.

possess enzyme activities (deacetylation, demethylation) that condense the chromatin, making it inaccessible to the transcriptional machinery (RNA Polymerase II and associated transcription factors). Fig. 3b shows the changes in the promoter region in response to the presence of ligand. Ligand binding promotes the dissociation of co-repressor proteins from PPAR and RXR and favors association with co-activator proteins. Co-activator proteins possess enzyme activities (acetylation and methylation) that decompact chromatin, making it accessible for binding by the transcriptional machinery. Importantly, the transcriptional activity of a gene is the result of the integration of multiple physiological signals (developmental, nutritional (fasting or fed), hormonal, or cell-specific) converging at the gene promoter.

Physiological Functions of PPARs

The tissue-specific patterns of expression of the PPAR isotypes suggested that these proteins have distinct physiological roles, and this was further supported when each was specifically disrupted in mouse gene knockout models.

PPAR α

Tissue-Specific Expression. In adult rodents, PPAR α is expressed in liver, kidney, intestine, heart, skeletal muscle, retina, adrenal gland, and pancreas. In adult human, PPAR α is expressed in the liver, heart, kidney, large intestine, skeletal muscle (mostly slow-twitch oxidative type I fibers), and in cells of atherosclerotic lesions (endothelial cells, smooth muscle cells, and monocytes/macrophages). Therefore, regardless of

species, PPAR α expression is highest in tissues with high rates of fatty acid catabolism.

Natural PPAR α Ligands. Multiple molecules endogenous to the cellular environment have been shown to bind and activate PPAR α *in vitro*, but it remains unclear whether there is a single predominant PPAR α ligand *in vivo*. Molecules demonstrated to bind PPAR α include mono- and polyunsaturated fatty acids and various fatty acid-derived eicosanoids. Interestingly, mice without a functional gene encoding fatty acid synthase (FAS) exhibit a phenotype very similar to fasted mice lacking a functional PPAR α gene: defects in fatty acid oxidation and severe hypoglycemia. These defects in FAS-deficient mice are corrected with treatment of PPAR α agonist, suggesting that FAS activity may regulate PPAR α activity by providing newly synthesized ligand and that, as ligands and activators of PPAR α , fatty acids derived from adipose depots may not be equivalent to fatty acids synthesized *de novo* via FAS.

Insight from the PPAR α Knockout Mouse. PPAR α -deficient adult mice are viable, fertile, and healthy, indicating that PPAR α is not essential for embryonic development. When adult PPAR α ^{-/-} mice are treated with fibrates, the characteristic response to PP is abolished, with no liver weight increase, no increase in

the size or number of peroxisomes, and no transcriptional induction of genes involved in peroxisomal β -oxidation. Furthermore, lipid droplets deposited in hepatocytes suggest a key role for PPAR α in maintaining normal lipid homeostasis. In the past decade since the PPAR α ^{-/-} mouse model was first reported, a wealth of evidence has confirmed the essential role of PPAR α in fatty acid catabolism via peroxisomal and mitochondrial β -oxidation and in **lipoprotein metabolism**. Table 1 summarizes the role of PPAR α in hepatic lipid metabolism, lipoprotein metabolism, and atherosclerosis.

PPAR γ

Tissue-Specific Expression. In the adult rodent, PPAR γ is expressed in brown and white adipose tissue, and at lower levels in intestine, retina, skeletal muscle, and lymphoid organs. In human, PPAR γ is most abundantly expressed in white adipose tissue and at lower levels in skeletal muscle, the heart, and liver, but not in lymphoid tissues, although PPAR γ has been identified in macrophages in human atheromas.

Natural PPAR γ Ligands. Endogenous ligands demonstrated to bind and activate PPAR γ *in vitro* include unsaturated fatty acids and their derivatives such as prostaglandin J2 (15-deoxy- Δ 12,14-PGJ2). Consistent

Peroxisome Proliferator-Activated Receptors. Table 1 PPAR α target tissues, cellular effects, and physiological effects

Tissue	Cellular effects	Physiological effects
Liver	↑ Fatty acid uptake into hepatocytes	↓ VLDL production
	↑ Peroxisomal and mitochondrial fatty acid β -oxidation	↑ Clearance of TG-rich lipoproteins
	↑ Transcription of lipoprotein lipase (LPL) gene	↓ Circulating TG concentrations
	↓ Synthesis of apoC-III (inhibitor of LPL)	↓ Proportion of small, dense LDL-C particles
	↑ Transcription of apoA-I and apoA-II genes (components of HDL)	↑ Proportion of large LDL-C particles (more efficiently cleared by the LDL-receptor)
		↑ Circulating HDL-C concentrations
		↑ Reverse cholesterol transport (delivery of excess cholesterol in the peripheral tissues to the liver via HDL-C for hepatic excretion into the bile)
		↓ Hepatic markers of inflammation (CRP, fibrinogen, etc.)
Pancreas	↑ Fatty acid oxidation	Protection of pancreatic β -cells from lipotoxicity
	Potentialiation of glucose-stimulated insulin secretion	
Atherosclerotic lesion	↓ Monocyte recruitment	↓ Progression of atherosclerosis
	↓ Expression of adhesion molecules	
	↓ LDL oxidation	
	↑ Cholesterol efflux from foam cells	
	↓ Inflammation and smooth muscle cell proliferation	

Abbreviations: apoC-III, apolipoprotein C-III; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; CRP, C-reactive protein; VLDL, very low density lipoprotein; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

with the other PPAR isotypes, it is unclear whether a predominant bioactive ligand exists *in vivo*.

Insight from the PPAR γ Knockout Mouse. Deletion of the PPAR γ gene results in embryonic lethality. At day 9.5 of gestation or earlier PPAR $\gamma^{-/-}$ embryos are viable, but no PPAR $\gamma^{-/-}$ embryos are viable after E9.5. This embryonic lethality is due to defects in the placenta leading to cardiac defects. When the placenta defect was corrected by genetic placental reconstitution, one PPAR $\gamma^{-/-}$ pup survived to term and demonstrated marked abnormalities in lipid deposition (lipodystrophy and fatty liver) and hemorrhages leading to death during the first week of life. Consistent with the tissue expression of PPAR γ , the PPAR γ -null mouse indicated that the receptor has essential roles in maintaining normal adipose tissue development. Subsequent studies have shown that PPAR γ plays a pivotal role in adipocyte differentiation and metabolism, insulin sensitivity, inflammation, and atherosclerosis. More recently PPAR γ has been implicated as both a tumor suppressor and a tumor promoter. The diverse functions of PPAR γ are summarized in Table 2.

PPAR δ

Tissue-Specific Expression. In the adult rat, PPAR δ is expressed in all tissues examined, and often at levels higher than PPAR α and PPAR γ . In human, PPAR δ is ubiquitously expressed, with higher expression in the digestive tract and placenta.

Natural PPAR δ Ligands. Similar to the other PPAR isotypes, endogenous ligands shown to bind and activate PPAR δ *in vitro* include fatty acids and eicosanoids, and it is not clear whether PPAR δ is preferentially bound and activated by specific ligands *in vivo*. However, fatty acids derived from very low density lipoprotein (VLDL) particles have been shown to activate PPAR δ target genes in a receptor-dependent manner, suggesting that fatty acids delivered to tissues via VLDL may possibly bind and activate PPAR δ *in vivo*.

Insight from the PPAR δ Knockout Mouse. For many years the physiological relevance of PPAR δ was unclear, in large part due to its ubiquitous expression which led to the assumption that this isotype served a “general housekeeping” role. However, deletion of the PPAR δ gene results in frequent (>90%) midgestation lethality due to defects of the placenta. All adipose depots are substantially decreased in adult PPAR δ -deficient animals; abdominal fat, interscapular brown fat, mesenteric fat, and adipose stores associated with internal organs are all underdeveloped compared to wild type animals through a decrease in adipocyte number. The subcutaneous fat layer, on the other hand, is approximately twofold thinner in PPAR δ -null animals by a reduction in both adipocyte number and size. The PPAR $\delta^{-/-}$ mouse provided evidence that, similar to the PPAR α and PPAR γ isotypes, PPAR δ has an important role in normal adipose development

Peroxisome Proliferator-Activated Receptors. Table 2 PPAR γ target tissues, cellular effects, and physiological effects

Tissue	Cellular effects	Physiological effects
Adipose	Adipocyte differentiation	↑ Proportion of small, insulin sensitive adipocytes
	Adipose remodeling; increase in subcutaneous adipose depots; decrease in visceral depots	↓ Proportion of hypertrophic, insulin resistant adipocytes
	↑ Transcription of lipoprotein lipase, fatty acid transport protein, and oxidized LDL receptor	Redirects fat for storage from visceral to subcutaneous adipose depots
	↑ Transcription of PPAR γ -coactivator 1 α (PGC-1 α)	↑ Expression of genes promoting storage of fat in subcutaneous adipocytes
	↑ Insulin sensitivity ^a	↑ Mitochondrial biogenesis, increasing fatty acid oxidation in adipocytes
	Altered expression and secretion of adipose tissue-derived factors thought to affect insulin sensitivity: adiponectin, TNF- α , IL-6, etc.	↑ Insulin sensitivity and glucose-lowering
Macrophage/atherosclerosis	↑ Reverse cholesterol transport from foam cells	↓ Progression of atherosclerosis
	↓ Expression of multiple inflammatory proteins	
Multiple cancers (colon, breast, prostate and others)	Tumor suppressor effects	To be determined
	Colon cancers with APC gene mutations: tumor promoter effects	

^aThe exact mechanism by which PPAR γ ligands affect insulin resistance (improved glucose uptake by peripheral tissues, most notably skeletal muscle) remains unclear.

Abbreviations: LDL, low density lipoprotein; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; APC, adenomatous polyposis coli tumor suppressor.

Peroxisome Proliferator-Activated Receptors. Table 3 PPAR δ Target tissues, cellular effects, and physiological effects

Tissue	Cellular effects	Physiological effects
Adipose	↑ Fatty acid transport and oxidation	↑ Oxidative disposal of fatty acids
	↑ Oxidative metabolism, mitochondrial respiration and thermogenesis	↓ Storage of excess fat in adipocytes ↓ Weight gain
Skeletal muscle	↑ Fatty acid transport and oxidation	↑ Oxidative disposal of fatty acids
	↑ Oxidative metabolism, mitochondrial respiration and thermogenesis	↓ Storage of excess fat in skeletal muscle
	Muscle fiber remodeling; increased proportion of oxidative slow-twitch fibers (often increased in response to endurance training)	↓ Weight gain ↑ Glucose uptake into muscle
Heart	↑ Fatty acid transport and oxidation	↑ Oxidative disposal of fatty acids
	Maintains fatty acid oxidation as the primary source of energy in the postnatal heart	Protects against situations where fatty acid oxidation decreases and glucose utilization increases e.g., sepsis
Macrophage/ atherosclerotic lesion	Anti-inflammatory effects ^a	
Additional PPAR δ effects	↑ Serum levels of apoA-I and apoA-II (components of HDL-C)	↑↑ Circulating HDL-C concentrations ^b
	↓ Expression of <i>Niemann-Pick C1-like-1 (NPC1L1)</i> gene (a key mediator of intestinal cholesterol absorption)	↓↓ Circulating TG and LDL-C concentrations ^b
		↓ intestinal cholesterol

^aContradictory results from preliminary studies suggest that the role of PPAR δ in atherosclerosis requires further investigation.

^bThe exact mechanism by which PPAR δ ligands potentially raise HDL-C levels and lower TG and LDL-C levels remains unclear.

Abbreviations: apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; LDL-C, low density lipoprotein cholesterol.

and in lipid homeostasis. Table 3 summarizes the functions of PPAR δ .

Additional Functions of PPARs

CNS Development

The expression of all three PPAR isotypes peaks in the rat central nervous system between days 13.5–18.5 of gestation, and while expression of both PPAR α and PPAR γ decline post-natally, expression of PPAR δ remains high (except for the retina, where all three isoforms are expressed in the adult rodent). An important role for PPAR δ in CNS development is underscored by the occurrence of defective myelination in the PPAR δ -null mouse.

Epidermal Maturation and Wound Healing. All three PPAR isotypes are expressed during epidermal maturation; each isotype has a specific pattern of expression in regard to development and the various layers of the epidermis. An important role for PPAR δ in the development and/or maintenance of normal skin health is indicated by the presence of defective wound healing in the PPAR δ -null mouse.

Drugs

Table 4 summarizes the physiological functions of the PPAR isotypes and their synthetic ligands.

PPAR α

Fibrates such as fenofibrate (Tricor[®]) and gemfibrozil (Lopid[®]) are FDA-approved for the treatment of dyslipidemia, and their primary effects are to lower triglycerides (TG) and raise high density lipoprotein cholesterol (HDL-C, the “good” cholesterol). Fibrates may also moderately decrease low density lipoprotein cholesterol (LDL-C, the “bad” cholesterol). Several clinical trials have demonstrated that fibrates slow the progression of atherosclerosis and reduce the risk of cardiovascular morbidity and mortality, particularly in patients with features of insulin resistance (overweight or obesity, hyperinsulinemia, hyperglycemia, or atherogenic dyslipidemia).

PPAR γ

Many synthetic ligands have been shown to bind and activate PPAR γ , but two molecules belonging to the thiazolidinedione (TZD) class have been FDA-approved for the treatment of type 2 diabetes: pioglitazone (Actos[®]) and rosiglitazone (Avandia[®]). TZDs are a class of ►oral anti-diabetic drugs, and several clinical trials have demonstrated that both pioglitazone and rosiglitazone reduce insulin resistance and improve glucose control in patients with type 2 diabetes. Although the exact mechanism by which TZDs improve insulin sensitivity remains unclear, one

Peroxisome Proliferator-Activated Receptors. Table 4 Summary of the physiological actions of PPAR isotypes and their synthetic ligands

PPAR isotype	Tissues of highest expression	Primary physiological effects	Endogenous ligands	Synthetic ligands	Associated disease state/indication
PPAR α	Liver, heart, skeletal muscle, atherosclerotic lesions	TG- and LDL-C-lowering and HDL-C-raising; re-directs excess cholesterol from the peripheral tissues to the liver for excretion into the bile via HDL-C; slowed progression of atherosclerosis	Fatty acids, eicosanoids: (fatty acids derived from FAS?)	Fibrates; fenofibrate (Tricor [®]), genfibrozil (Lopid [®])	Dyslipidemia
PPAR γ	White adipose tissue, atherosclerotic lesions	Insulin-sensitizing and glucose-lowering; re-directs TG from non-adipose tissues and visceral adipose depots for storage in subcutaneous adipose tissue; slowed progression of atherosclerosis	Fatty acids, eicosanoids	Thiazolidinediones; pioglitazone (Actos [®]), rosiglitazone (Avandia [®])	Type 2 diabetes, (insulin resistance, metabolic syndrome)
PPAR δ	Ubiquitous	Potent TG- and LDL-C-lowering and potent HDL-C-raising; increased oxidative disposal of fatty acids in adipose and skeletal muscle; thermogenesis; weight loss	Fatty acids, eicosanoids; (fatty acids derived from VLDL particles?)	GW501516 <i>currently in Phase II clinical trials</i>	Dyslipidemia, obesity? atherosclerosis?

Abbreviations: TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; FAS, fatty acid synthase; VLDL, very low density lipoprotein.

US product information: Actos[®]: ▶ <http://www.Actos.com>; Avandia[®]: ▶ <http://www.Avandia.com>; Tricor[®]: ▶ <http://www.tricortablets.com>; Lopid[®]: ▶ <http://www.pfizer.com>.

potential mechanism is that TZDs increase plasma levels of adipose-derived factors (▶ **adipokines**) associated with improved insulin sensitivity while decreasing plasma levels of several adipokines associated with insulin resistance.

PPAR δ

Although no PPAR δ -specific ligands are currently FDA-approved, GW501516 is a compound being developed jointly by GlaxoSmithKline and Ligand Pharmaceuticals. This compound is currently in Phase II trials for the treatment of dyslipidemia.

In summary, the PPARs coordinately function to decrease lipid content in non-adipose tissues by a variety of mechanisms, and in doing so improve insulin sensitivity and glucose control and slow the progression of atherosclerosis. Although these functions of PPARs are currently the best understood, additional roles of PPAR isotypes in other diverse physiological processes is being investigated. As additional roles are defined, PPAR isotypes will continue to be important molecular targets for identifying ligands (drugs) with potential applications to reproduction and fertility, myelination and normal development of the nervous system, and cell-cycle control, skin biology, wound-healing and cancer.

- ▶ **Nuclear Receptors**
- ▶ **Drug-Receptor Interaction**
- ▶ **Inflammation**
- ▶ **Atherosclerosis**
- ▶ **Cell Cycle Control**
- ▶ **Diabetes Mellitus**
- ▶ **Retinoids**
- ▶ **Lipoprotein Metabolism**
- ▶ **Antidiabetic Drugs other than Insulin**
- ▶ **Adipokines**

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Peroxynitrite

A potent oxidant that is formed by NO reacting with superoxide anions.

► Nitric Oxide

Pertussis Toxin

Pertussis toxin is produced by the bacterium *Bordetella pertussis*. It covalently modifies G-proteins of the G_i/G_o family (transfer of a ADP-ribose moiety of NAD onto G-protein α -subunits). ADP-ribosylated G-proteins are arrested in their inactive state and, as a consequence, functionally ‘uncoupled’ from their respective effectors. Examples for pertussis toxin-sensitive cellular responses include the hormonal inhibition of adenylyl cyclases, stimulation of K^+ channels, inhibition of Ca^{2+} channels and stimulation of the cGMP-phosphodiesterase in retinal rods.

- Bacterial Toxins
- Somatostatin
- Transmembrane Signaling
- Heterotrimeric GTP-Binding Proteins

PEST Sequences

PEST sequences are protein regions with a high content of the amino acid regions proline (P), glutamate (E), serine (S), and threonine (T). These sequences often serve as destruction signals recognized by the ubiquitin proteasome system (UPS). Phosphorylation of serine or threonine residues in these regions appears to be the trigger for recognition by certain SCF-RING ubiquitin (Ub) ligases.

► Ubiquitin/Proteasome System

PGI₂

Prostacyclin.

► Prostanoids

P-Glycoprotein

The permeability-glycoprotein (P-gp) is the first ABC-transporter identified in human. It is also termed ABCB1 or MDRA and eliminates a variety of structurally unrelated compounds from the cell. P-gp is highly expressed in certain cancer cells thereby conferring resistance against many anticancer drugs. P-gp is also expressed in normal tissues like the blood–brain barrier, the intestine, or the blood–placenta barrier, where it protects the brain, the body and the growing foetus against toxic compounds.

- MDR-ABC Transporters
- ABC-Transporter

PH Domain

Synonyms

Pleckstrin homology

Definition

Domains consisting of about 120 amino acid residues. Important for localizing target proteins to the plasma membrane.

► Adaptor Proteins

PH-Sensing Receptors

Group of Rhodopsin – like G protein-coupled receptors comprising GPR4, GPR65 (T cell death-associated gene 8, TDAG8), and GPR68 (ovarian cancer G protein-coupled receptor 1, OGR1). These receptors are activated at neutral to slightly acidic extracellular pH, and likely implicated in pH homeostasis, bone metabolism, angiogenesis, inflammation.

► Proton-Sensing GPCRs

Phage

► Bacteriophage

Phagocyte/Phagocytosis

Phagocytes are a group of cells that may engulf and internalise antigens, pathogens or apoptotic cells and destroy them.

- ▶ Apoptosis
- ▶ Inflammation
- ▶ Immune Defense

Pharmacodynamics

Pharmacodynamics describe the effects elicited by a drug in the body.

- ▶ Pharmacogenetics
- ▶ Pharmacokinetics

Pharmacogenetics

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Definition

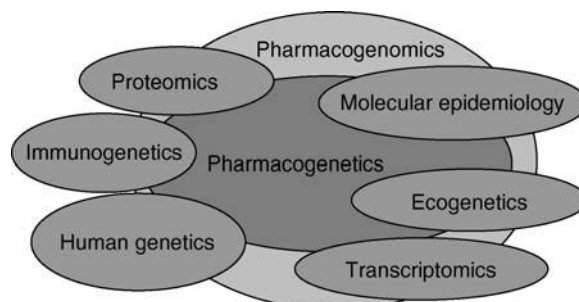
Pharmacogenetics is the study of genetically determined inter-individual variation within one species with respect to the response to drugs. Inherited genetic variations with frequencies above one percent are termed ▶ **polymorphisms**. In the human genome, there are more than three million polymorphisms. Some of these polymorphisms are the cause why some people do not respond to drugs or do respond extremely heavily or suffer from adverse events to drugs. Pharmacogenetics also includes the impact of genetic variability for an organism's response to any external chemical, physical or microbial influence (▶ **ecogenetics**), and some genetic polymorphisms affect internal processes of an organism. Therefore, pharmacogenetics may also provide explanations of individual differences in the ▶ **susceptibility** for a number of diseases. In contrast to inherited disease genes studied in human genetics, pharmacogenetic polymorphisms are not closely related

to disease and typically the effect of genetic polymorphism become only apparent upon exposure to drugs or other conditions (▶ **gene–environment interactions**).

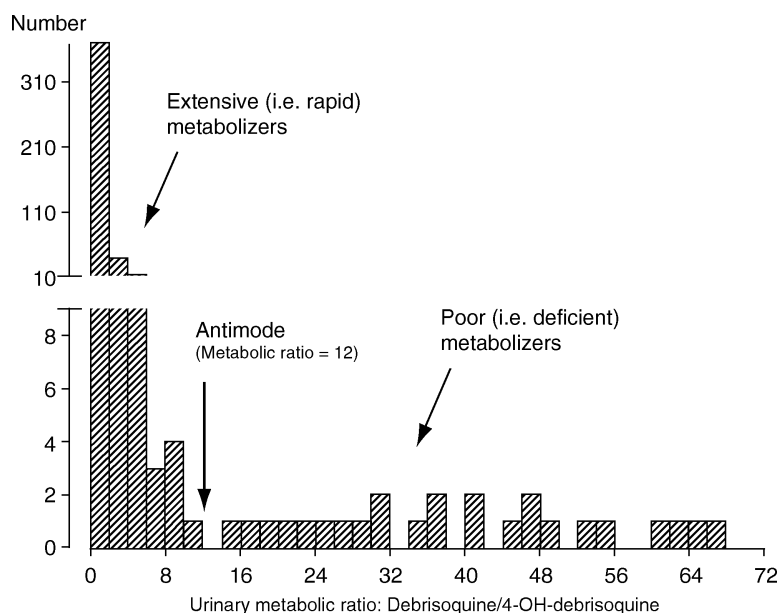
Basic Mechanisms

Pharmacogenetics is a rapidly evolving field of research [1–5]. Fig. 1 illustrates the relationships of pharmacogenetics with other related disciplines. Historically, pharmacogenetic research started with the measurement of the ▶ **phenotype**, which is the apparent variability in structures or functions of one species (phenotyping). For instance, carriers of the deficiency of the drug metabolizing enzyme ▶ **cytochrome P450 2D6** were identified as persons who did not excrete significant amounts of metabolites of drugs like debrisoquine or sparteine (i.e., they showed the deficient or ▶ **poor metabolizer** phenotype). The frequency distribution of this phenotype is illustrated in Fig. 2. For a monogenic determined polymorphism, the phenotype can typically be classified into two groups (▶ **bimodal distribution**) or three groups (▶ **trimodal distribution**). The values separating the two or three groups is termed ▶ **antimode**. For autosomal genes, a bimodal distribution would correspond to a dominant mode of inheritance. Trimodal distributions are more frequent in pharmacogenetics that mostly correspond to a ▶ **codominant** mode of inheritance. Nowadays, genetic polymorphisms are identified by molecular genetic analysis of genomic DNA (genotyping). The analysis of the correlation between a DNA variation and the phenotype (genotype–phenotype correlation) is an essential topic of current ▶ **functional genomics** research.

Essential to the definition of Pharmacogenetics is the term genetic polymorphism. It is extrapolated that there are at least three million genetic polymorphisms in the human genome. Historically, a genetic polymorphism was defined as a genetic variation with a population frequency of 1% and above, but the larger inter-ethnic variation of population frequencies makes a strict definition based on such frequencies impractical. The most common molecular type of polymorphism is the



Pharmacogenetics. Figure 1 Relationship between Pharmacogenetics and other disciplines illustrated using a Venn diagram.



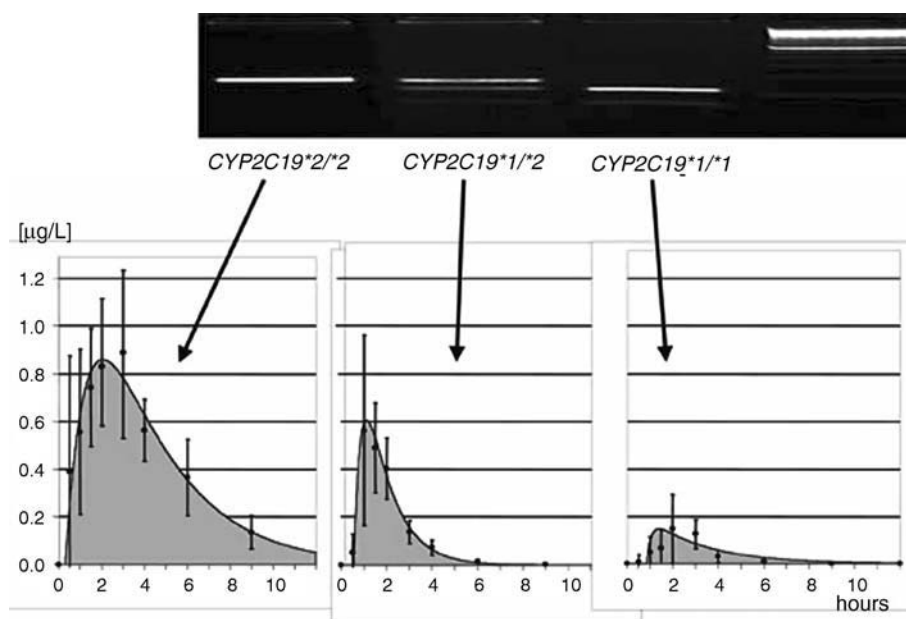
Pharmacogenetics. Figure 2 Cytochrome P450 2D6 (*CYP2D6*) polymorphism as a prototype for a pharmacogenetic polymorphism. The activity was measured using the drug debrisoquine as a test drug and measuring the urinary excretion of the test drug and its 4'-hydroxylated metabolite. The bimodal frequency distribution of *CYP2D6* in a human population ($N = 454$) is shown with the rapid or so-called extensive metabolizers on the left side of the antimode and the deficient or so-called poor metabolizers on the right side. Poor metabolizers are unable to generate significant amounts of the 4'-hydroxylated metabolite as indicated by the fact that their metabolic ratio is at least 12 corresponding to 12-times more parent drug than metabolite.

► **single nucleotide polymorphism (SNP)**. SNPs are classified according to their location in the coding region of exons as either synonymous SNPs (no change of the amino acid sequence) or nonsynonymous SNPs (change of the amino acid sequence). Most SNPs are localized outside the protein coding sequences. Many of these noncoding SNPs may be absolutely silent, but some of these polymorphisms have significant effects on gene transcription or splicing. Other molecular types of genetic variation include insertions or deletions of one or several nucleotides or even entire genes. Some polymorphisms consist of duplications or higher number amplifications of entire functional genes (for instance, the gene duplication of *CYP2D6* results in a phenotype with very extensive expression of the enzyme and ultra-rapid biotransformation of *CYP2D6* substrates). Another distinct type of polymorphisms are the so called variable number of tandem repeat polymorphisms (VNTR) where a segments of repetitive DNA sequence exit with inter-individually variable number of repeats (also termed microsatellites).

Some authors use the term ► **mutation** as a synonym for genetic polymorphism. However, it is recommended to reserve the term mutation for genetic variations acquired within the life span of an organism such as those mutations acquired in tumor tissues during multi-step carcinogenesis.

Within a chromosome, genetic polymorphisms are inherited in a linked fashion and on average, the more closely two polymorphisms are located to each other the more tightly they are linked, but polymorphisms even located one base-pair apart may not be linked. The specific combination of genetic polymorphisms within one chromosome is termed ► **haplotype**. Compilations of genetic polymorphisms such as those for human cytochrome P450 enzymes (► www.imm.ki.se/CYPalleles) or those for ► *N*-acetyltransferases (► <http://www.louisville.edu/medschool/pharmacology/NAT.html>) define the polymorphic alleles as much as possible as haplotypes of linked SNPs or other types of variants. Theoretically, medical studies on the function of polymorphisms should always be based on haplotypes, but complete experimental identification of haplotypes from the diploid cells is almost unfeasible with current technologies.

Genetic polymorphisms may affect the fate of a drug within the body of an organism (► **pharmacokinetics**) and genetic polymorphisms may affect how the drug is acting on the molecular targets of the organism (► **pharmacodynamics**). Polymorphisms relevant for pharmacokinetic variability are in drug transporters relevant for active or facilitated transport through biological barriers and in the enzymes catalyzing drug biotransformation. The effect of polymorphisms in the enzymes of drug biotransformation has been the main



Pharmacogenetics. Figure 3 Illustration of the genotype–phenotype correlation on the example of the cytochrome P450 2C19 polymorphism. The upper part illustrates a molecular analysis of this polymorphism using PCR and restriction fragment analysis resulting in a loss of the restriction site in carriers of the nonfunctional variant *2. The genotype *CYP2C19*2/*2* indicates poor metabolizers of substrates of the enzyme CYP2C19 and as shown below, the area under the concentration time curve of them is about 15-fold higher compared to homozygous extensive metabolizers (genotype *CYP2C19*1/*1*). The phenotype of the heterozygotes is inbetween corresponding to a codominant mode of inheritance typical for polymorphisms in drug metabolizing enzymes.

focus of pharmacogenetic research in the past. Fig. 3 illustrates on the example of the ►cytochrome P450 2C19 polymorphism the genotype–phenotype correlation. In this example, the exposure of the human body to omeprazole differs about 15-fold depending on the cytochrome P450 polymorphism as expressed as the area under the blood plasma concentration time curve (►AUC) of the drug. In general, the rapid metabolizers may be prone to ineffective action of the drug and the deficient metabolizers (the so-called poor metabolizers) may be prone to adverse effects due to overdosage. The medical impact differs depending on the therapeutic index and other properties of each drug; in the given example of omeprazole, low efficacy in rapid metabolizers was identified as a problem.

There are only very few genes which do not carry any polymorphisms. And there exists even a significant number of polymorphic genes that are not expressed at all in part of the population due to genetic polymorphisms. Table 1 summarizes a few selected polymorphisms with their functional and medical impact.

Pharmacological Relevance Individualization of Drug Treatment

If the genotype–phenotype correlation is sufficiently high, an individual's phenotype can be predicted by the

genotype. Thus, genotyping can be used to predict in part an individual's response to specific drugs. A drug therapy optimized according to a number of genetic variants is likely to be more efficacious and will produce fewer or less severe adverse effects compared with current drug therapy. It is anticipated that in future nonresponders to drugs who are explained by ultrarapid drug biotransformation or by their less reactive receptors will receive higher drug doses or alternative drugs. Heavy responders to drug due to genetic receptor variation or due to poor drug biotransformation will receive lower doses or alternative drugs. In particular, subjects prone to idiosyncratic adverse drug reactions that are due to immunogenetic polymorphisms, impaired detoxification of reactive metabolites or other genetic variations will not be treated with the drugs which may be dangerous for this subgroup.

In current daily medical practice, the concept of pharmacogenetic genotyping prior to drug treatment is not routinely used with a few exceptions. At some clinics, phenotyping or genotyping ►thiopurine S-methyltransferase (Table 1) is performed prior to therapy with azathioprine and the doses are adjusted accordingly. Currently routine application of pharmacogenetic testing in medicine is limited in part due to lack of knowledge about application and impact of

Pharmacogenetics. Table 1 Selected genetic polymorphisms and their medical impact

Enzyme	Functional effects of polymorphism and frequency	Examples for the medical impact
Glucose-6-phosphate dehydrogenase	Low or absent enzyme activity in about 10% of African populations.	Hemolysis following intake of a number of drugs which have electrophilic reactive metabolites, but also, carriers of this enzyme deficiency have a partial protection from malaria.
Pseudocholine esterase	Low or absent activity in 0.05% of Caucasian populations.	Prolonged action of succinylcholine and mivacurium.
Paraoxonase (PON1)	Low activity in carriers of the glutamine192 variant with a population frequency of about 50% for the homozygous glutamine genotype in Caucasians.	Carriers of low activity may show higher lipid peroxidation and higher toxicity from organophosphorothioate insecticides.
NADPH:quinone oxido-reductase 1	Pro187Ser variant occurring with about 5% frequency is functionally almost completely deficient.	Impaired activity associated with benzene toxicity and cancer chemotherapy induced leukemia.
Cytochrome P450 2C9	Low activity in about 10% (heterozygotes) and very low activity in about 0.8% (homozygotes) of Caucasian populations.	Prolonged action of several CYP2C9 inactivated drugs like phenytoin, tolbutamide, ibuprofen, or S-warfarin.
Cytochrome P450 2C19	Deficient activity in about 3% of Caucasian populations and in about 20% of Asian populations.	Prolonged action of several CYP2C19 inactivated drugs like omeprazole or diazepam in the poor metabolizers.
Cytochrome P450 2D6	Extremely high activity in about 2% of Caucasian populations and completely deficient activity in about 7%.	Inefficiency in ultrarapid metabolizers and extremely heavy effects in poor metabolizers for more than 50 drugs. A few drugs requiring bioactivation by CYP have low efficacy in poor metabolizers (example: codein is activated to morphine via CYP2D6).
N-acetyltransferase 2	Low activity in about 60% of Caucasian populations.	High incidence of adverse events from the drug isoniazide in slow acetylators.
Glutathione S-transferase M1	Deficient activity in about 50% of Caucasians.	Carriers of the deficiency may have a slightly increased risk for a number of smoking-related cancers.
Thiopurine S-methyltransferase	Low activity in about 10% of Caucasians and deficient activity in about 0.4%.	High incidence of severe adverse events from azathioprine and 6-mercaptopurine in carriers of low activity.
β -Adrenergic receptor 2	Amino acid variants appear to be associated with receptor function and agonist induced downregulation.	Some variants may predispose to some types of asthma and modulate action of β -2-adrenergic drugs.
Factor V Leiden	Factor V Leiden refers to a Arg506Gln replacement which occurs with about 5% frequency in the heterozygous form in Caucasians. The variant is inactivated approximately ten times slower than normal factor V.	About five-fold increased risk for thrombosis in heterozygous carriers of the glutamine variant. Extremely increased risk in homozygous carriers and in heterozygotes taking oral contraceptives. On the other hand, possibly protection from severe blood loss during accidents, surgery or child birth.
5-Lipoxygenase	A VNTR polymorphism in 100 bp upstream from the ATG start codon is associated with transcription efficiency.	Differences in response to 5-lipoxygenase inhibitors and leucotriene receptor antagonists.
β -Adrenergic receptor 2	Amino acid variants moderately associated with receptor function and agonist induced downregulation.	Some variants may predispose to some types of asthma and modulate action of β -2-adrenergic drugs.
Cysteine-cysteine chemokine receptor 5 (CCR5)	A 32-bp deletion with a population frequency of about 0.1 in Caucasians results in truncated nonfunctional receptor.	Carriers of this variant are partially protected from HIV infection, particularly the homozygous carriers.
G protein β -3 subunit	Truncated protein with increased signal transduction in carriers of the 825T allele.	Associated with hypertension and with the response to thiazide diuretics.

pharmacogenetics among physicians and in part due to the limited availability of rapid genotyping methods. In some instances only moderate specificity or sensitivity depending on the relevant gene and the clinical problem may also be a problem. For instance, poor biotransformation of substrates of CYP2C19 or CYP2D6 can be predicted with a 100% specificity and positive predictive value, thus, low doses can be prescribed to poor metabolizers with sufficient certainty. But sensitivity and negative predictive value are less than 100% since other factors like drug–drug–interactions, rare unidentified polymorphisms or unidentified liver disease may also result in poor metabolism.

Pharmacogenetics as a Compound Selection Tool in Drug Development

In drug development the impact of frequent genetic polymorphisms particularly in genes involved in drug biotransformation is routinely studied by *in vitro* methods and by clinical trials in humans. Preference is given to those drugs not significantly affected by genetic polymorphisms, but existing effects of genetic polymorphisms is not necessarily a knockout criterion if the drug has specific properties not available with other analogues of that class. In addition, pharmacogenetic testing during clinical drug trials may help to reduce variability and thus to more specifically identify the effects of a certain drug in smaller numbers of subjects.

Pharmacogenetics as a Discovery Tool for Mechanisms of Diseases and New Therapeutic Principles

The existence of naturally occurring variations in almost all genes in the human population provides a way to identify the genes involved in disease pathogenesis. This may be particularly helpful in those diseases or adverse drug effects where the mechanisms are unknown. By correlating genetic polymorphisms in possibly related genes (candidate gene approach) or in the entire genome (gene mapping approach) one may be able to identify genes and their polymorphisms that are causative for that disease. This may then allow to design new drugs or other therapeutic principles targeted at these genes.

► **Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes**

► **P450 Mono-oxygenase System**

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Pharmacogenomics

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Synonyms

Broader concept of pharmacogenetics

Definition

The conceptual difference between “pharmacogenomics” and “pharmacogenetics” is ambiguously justified, but may be easily compared with the differential concepts of mono/oligogenic and polygenic disease. In contrast to “pharmacogenetics,” which is more or less dedicated to study the impact of a few polymorphic drug-related pharmacokinetic/dynamic targets (drug-metabolizing enzymes, receptors, etc.), the concept of “pharmacogenomics” is applied for the study of an ensemble of drug-related pathways (inflammatory pathways for atherosclerotic disease or the renin–angiotensin system for essential hypertension for example), more complex system biologic pathways (hypothesis-driven) or genome-wide disease- or drug-response susceptibility (nonhypothesis driven).

Principal Goals

The anticipated goal of both disciplines is to help ensuring the “right” drug selection, optimizing choice of drug combinations and dosing, improve drug efficacy and to minimize drug toxicity. This should finally result in the establishment of more personalized treatment concepts for ► **complex disease**.

Basic Mechanisms Prerequisites

The prerequisite of the relatively new disciplines pharmacogenetics/pharmacogenomics is that individuals

effectively differ in their genomic nucleotide sequences (nucleotide diversity represented by differential sequence order of nucleotides G, A, T, and C, throughout the genome), representing a complex interindividually variant genetic make-up. In a usual genetic scan, single-base substitutions (►single-nucleotide polymorphisms [SNPs]) are the most common form of genetic variation (85%) followed by insertion/deletion variations (8%), repeat polymorphisms (6%), and others (1%). A gene is usually composed of 5'-flanking including a distal, proximal, and core promoter and 5'-untranslated portion, exonic, intronic, 3'-untranslated, and 3'-prime (both 3'-flanking) regions. Already in the late 1990s, from a systematic genetic scan of dozens of ►candidate genes (candidate genes are suspected of involvement in the disease process.) for cardiovascular disease (CVD) a considerable genetic diversity in a population of European ancestry has been observed [1]. The average level of nucleotide diversity (π) estimated from the pooled genes was about 3.7×10^{-4} , which means that two randomly chosen sequences from the population are expected to differ approximately every 2,700 bp. The average nucleotide diversity was 4.4×10^{-4} in the 5'- and 3'-flanking gene regions (1 variable site/2,300 bp) and 3.0×10^{-4} in the exonic regions (1 variable site/3,400 bp); these data were confirmed by subsequent studies in Caucasian samples. The lower sequence diversity observed in exonic than in the 5'- and 3'-flanking regions presumably reflected a more stringent selective pressure with respect to coding than to noncoding sequences.

Recent Advances

Celera Genomics and separately the international Human Genome Project consortium each described their first drafts of the genetic blueprint for a human in 2001, even when both teams combined samples from several individuals and created composite genomes that contained only half of a human's DNA. According to a recent study by Venters' group (October 2007), however, haploid genomes underestimate the amount of genetic variation between individuals by a factor of 5. The availability of almost the entire human genome sequence allows for the immediate access to specific target sequences across the human genome. The technologies to explore genetic sequences on a "genome-wide" scale and high-throughput genotyping methods literally exploded since then. ►Genome-wide approaches take advantage of the fact that alleles at nearby ►loci often show strong ►linkage disequilibrium (LD), which is coupled with observations that human recombination is concentrated into short hotspots (1–2 kb) that occur every 100–200 kb; as these recombination hotspots are often coincident with a breakdown of allelic association a few markers within each domain of strong association can be used to tag nearby variation. Choosing SNPs at set intervals across the genome will fail to capture local patterns of allelic association, therefore, the International

HapMap Project was founded in 2002, with the goal of mapping the structure of allelic association across the human genome (The International HapMap Consortium). High-throughput whole-genome genotyping of a few hundred thousand well-chosen SNPs should provide adequate power in most populations to detect single-locus associations for SNPs of moderate frequency and relative risk. For that purpose, an Affymetrix GeneChip Human Mapping 100K SNP set (100K set) has been launched in 2004. This 100K set included 116 204 SNPs, with a median and mean intermarker distance of 8.5 and 23.6 kb, respectively, with 92% of the genome within 100 kb of a SNP. As genome-wide association (GWA) studies are likely to require >100,000 SNPs, 500K sets have been published and genome-wide scans, identifying associations of common variants on chromosome 9p21 with MI in Caucasian populations have proven to being replicated by independent groups. This could also be applied for certain population samples allocated to pharmacological important entities such as "good responder," "non-responder," samples with significant side effects, also. All of these samples might have a common underlying genetic imprint that might only be detected in large-scale genomic applications. Beside the current versions of 500 or even 1000K chips, newly generated chips in that range might cover additional gene regions in a complementary setting to already existing ones. As most of the SNPs included in the 100K (and presumably in the 500K set either) are not coding ones (60% represent SNPs 2 kb outside a gene; 30% of SNPs are intronic ones; <1% are coding variants), once a ►candidate gene locus is proposed, extensive resequencing is warranted to actually identify the responsible variant.

The Concept of Pharmacogenomics

Some 50 years ago, it has been observed that the interindividual differences in drug response, besides age, gender, drug interaction, and the like, may also be assigned to inheritable factors. This was then referred to as "pharmacogenetics," which is the subject of another chapter within this encyclopedia. Genes that encode products, which interfere directly or indirectly (downstream or upstream of the drug target of interest) with drug action, may be polymorphic ("pharmacodynamogenetics"). An important example is the polymorphic beta-2-receptor (ADRB2). A glycine at codon 16 (Gly16) has been associated with increased agonist-promoted downregulation of ADRB2 as compared with Arg16. Glu27, on the other hand, has been shown to be resistant to downregulation when compared with Gln27, but only when coexpressed with Arg16. When compared with Gly16 homozygotes, Arg16 homozygotes were 5.3 times and Arg16 heterozygotes were 2.3 times more likely to respond to albuterol, respectively. Other investigators reported that an adenylyl cyclase type 9 (AC9) Ile772Met variant, an effector for airway ADRB2, altered albuterol responsiveness in the context of

concomitant inhaled corticosteroid administration, which renders the pharmacogenetic impact more complex.

Whereas pharmacogenetics deals with more or less well-defined genetic targets including drug metabolizing enzymes (CYP, NAT, TPMT, etc.) or specific pharmacological targets (receptors, counter receptors, ion channels, and the like), the term “pharmacogenomics” is applied for a more comprehensive and complex “genomic” analysis of genetic predisposition to drug response. In that respect, pharmacogenomics is aimed at identifying polymorphic pathways (system biologic) or even (yet unknown) genome-wide susceptibility to interindividual predisposition to drug response, which could also involve the search for susceptibility to complex disease.

► **Genotype–phenotype** (drug response) relationships are complex and the prerequisites to increase the likelihood of getting meaningful knowledge of these complex patterns is: (i) the sequence of all human genes; (ii) large freely accessible database accumulating genetic variants; (iii) new technologies allowing high throughput accurate genotyping at low cost; (iv) new bioinformatic and statistical tools allowing to deal with huge amount of data; (v) mega-studies connected to biobanks providing the necessary material for large-scale genotyping. Accounting for the simultaneous contribution of several genetic and nongenetic factors will be prerequisite for understanding the causal framework underlying complex drug-related traits.

Practical Considerations

In case an individual carries many different trait-“causing” or “-susceptibility” ► **alleles**, which qualifies the patient as “at-high-risk” for developing the disease; drug response to, for example, blood pressure lowering may also differ, depending on disease susceptibility. These individuals might require differential dose regimes and/or other drug combinations as usually prescribed without this additional knowledge of genomic susceptibility.

Another important “socioeconomic” problem is the occurrence of drug side-effects. In Germany, severe side-effects occur in >108,000 patients/year, leading to >>2,250 deaths/year with estimated direct costs of annually 400 million Euro. In its less severe characteristic, side-effects significantly affects adherence to continuous drug therapy (compliance); dry cough, for example occurs in approximately 5–10% of patients taking ACE inhibitors, but it definitely results in drug discontinuation. It has been proposed that there may be race- or ethnicity-related differences in the prevalence of cough attributed to ACE inhibitor therapy as it is significantly more prevalent in Blacks. This suggests that frequency and severity of drug side-effects may also be genetically (background) determined, and this would be again more important for life-threatening side-effects such as angioedema.

As these drug (side) actions may also involve polymorphic pathways, they are also amenable to gene

or genome scans. It is also conceivable that certain positive side-effects (antiinflammatory, anti-aggregatory actions of for example statins) only occur in those patients, which are susceptible to these actions, i.e., carriers of gain-of-function variations in the “inhibitory” pathway, and not in those with loss-of-function variations. With respect to the latter example, statins are known to inhibit isoprenoid generation, resulting in the inhibition of small GTP-binding protein activation such as Rho, Ras, and Rac, which then inhibits the activation of various downstream signaling pathways including mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinases (ERKs), p38MAPKs, c-jun N-terminal kinases (JNKs), and phosphatidylinositol 3-kinase (PI3K). Statins exert antiinflammatory properties by inhibiting transcription factors such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), but also as recently been reported via nuclear receptors such as peroxisome proliferator-activated receptor-α (PPARα) and PPARγ. These lipid-independent, diverse biological effects produced by the inhibition of isoprenoid synthesis have been collectively referred to as the statins’ pleiotropic effects and are thought to interfere with biological actions such as cell proliferation, oxidative stress, and inflammation and consequently the development of atherogenesis. Patients genetically high or low “functionally” susceptible to these pathways may differentially (and dose-dependently) respond to statin therapy. Other not yet known drug-related pathways within a given tissue or cellular context of interest may be identified by use of gene expression analyses or proteomic approaches. For the treatment of ► **essential hypertension** it has been convincingly shown that the response to diuretic therapy apparently depends on the presence of alpha-adducin genotypes (e.g., Gly460Asp). As the genetic impact on the phenotype was rather straightforward, a pharmacogenomic trial has recently been launched using a new drug target for essential hypertension treatment [2].

Other factors that may complicate dose–response relationships are gene–gene (epistasis; [3]) or gene–environment interactions (ecogenetic context, for example ► **allele**-dependent association with left-ventricular mass depending on salt-ingestion [4] or HDL-C level depending on alcohol consumption [5]). In the latter cases, the anticipated reduction of left ventricular hypertrophy or elevation of HDL-C level upon, for example, with ACE inhibitor or statin therapy, respectively, may not be attained with conventional drug doses (in either direction).

As pharmacogenetics and pharmacogenomics are not mutually exclusive, but even sometimes display overlapping features, both disciplines are aimed at better tailoring medical therapy for a given complex disease such as essential hypertension or other CVD. In the wake of new technologies to identify candidate region for whatever phenotype, more robust and complex bioinformatic analyses are necessary to

compute the observed genomic “effects” on drug response-related phenotypes to establish appropriate guidelines for a more individually tailored drug therapy. The ultimate goal is to (i) prescribe the most appropriate drug(s) for the patient, to (ii) individualize drug dosing, (iii) to optimize the number of prescribed compounds on a daily basis (convenience, adherence to drug therapy), and (iv) to reduce or even avoid side-effects and toxicity. To attain these goals, basic science has to be translated into clinical practice by use of practical clinical trials, outcome-oriented pharmacogenomic trials, and clinical trial regulatory measures.

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Pharmacokinetics

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Definition

In clinical life, as outlined in this essay, ►**pharmacokinetics (PK)** is used instrumental to improve drug therapy. For this purpose, pharmacokinetics must be presented in general and transmissible terms. The case of kidney failure gives one important example of how disease influences pharmacokinetics and how pharmacokinetics can be used to produce the same pharmacodynamic effect in such patients. The aim and end

will be an individualized drug ►**dose** by means of pharmacokinetic and pharmacodynamic principles.

Basic Mechanisms

Since the dose is given to produce an effect, it is obvious that pharmacokinetics is a required but not yet sufficient tool for drug dose adjustment ►**Pharmacokinetics (PK)** must be combined with ►**pharmacodynamics (PD)** to derive the individualized dose.

Drug => PK + PD => Individualized Dose

Also pharmacogenetics (PG) can be integrated into a common concept, and be figured as pharmacokinetic (PK) and pharmacodynamic parameters (PD) for the individual patient.

PG => PK + PD

One drug level (C_{indiv}) can be used with the means and standard deviation (SD) of population parameters (P_{pop}) as a priori knowledge for an individual parameter estimate using the Bayesian objective function.

$$\text{MIN} = \text{SIGMA}[(C_{\text{indiv}} - C_{\text{pop}})^2 / \text{SDc}^2] \\ + \text{SIGMA}[(P_{\text{pop}} - P_{\text{indiv}})^2 / \text{SDp}^2]$$

Based on therapeutic drug monitoring (►**TDM**) and the individual parameter estimates, every drug can be made applicable to every patient by individual dose adjustment.

Pharmacokinetics

According to the basic natural law, the time-dependent decrease ($-dA/dt$) is proportional to the actual amount of a drug (A) in the body. The proportionality constant is the elimination rate (Ke).

$$-dA/dt = K_e \cdot A$$

This differential equation can be integrated and the actual amount (A) is described by an exponential equation.

$$-dA/A = K_e \cdot t$$

$$\ln(A) - \ln(A_o) = -K_e \cdot t$$

$$\exp(\ln(A/A_o)) = \exp(-K_e \cdot t)$$

$$A = A_o \cdot \exp(-K_e \cdot t)$$

The ►**half-life (T_{1/2})** is the time to decrease the amount in the body to one half where (0.693 = ln2).

$$1/2 = \exp(-K_e \cdot T_{1/2})$$

$$\exp(K_e \cdot T_{1/2}) = 2$$

$$K_e \cdot T_{1/2} = \ln(2)$$

$$T_{1/2} = \ln(2)/K_e$$

$$T_{1/2} = 0.693/K_e$$

$$A = A_o \cdot \exp(-0.693 \cdot t/T_{1/2})$$

$$A = A_o \cdot 2^{(-t/T_{1/2})}$$

If an immediate effect is needed, a loading dose (D_{load}) must be given to administer the therapeutic amount ($D_{load} = A_0$). To maintain the drug effect, the maintenance dose (D) must be administered repetitively with the administration interval (τ).

$$D = A_0 - A$$

$$D = D_{load} * (1 - \exp(-0.693 * \tau / T_{1/2}))$$

Thus, for drug dosing, one only needs to know the loading dose or the standard dose and the elimination half-life.

Volume, Clearance, and Half-life

What can be measured in the body is the concentration (C) not the amount. The concentration is related to the amount (A) by the distribution ►volume (V_d).

$$C = A / V_d$$

$$C_0 = D / V_d$$

As the amount in the body decreases, the concentration decreases by the same law ($-dC/dt = K_e * C$), i.e., first-order kinetics resulting in an exponential function. The integral is the area under the concentration time curve (AUC).

$$C = C_0 \exp(-K_e * t)$$

$$AUC = C_0 [(1 / K_e) * \exp(-K_e * t_{oo}) - (1 / K_e * \exp(-K_e * t_0))]$$

$$AUC = C_0 / K_e$$

The ►clearance (Cl) is the constant parameter relating the change of the amount to the concentration ($-dA/dt = Cl * C$).

$$Cl = (-dA/dt) / C$$

$$Cl = D / AUC$$

From the above functions ($Cl = D / AUC$) and ($D = C_0 * V_d$) the fundamental equation can be derived (Fig. 1).

$$Cl = K_e * V_d$$

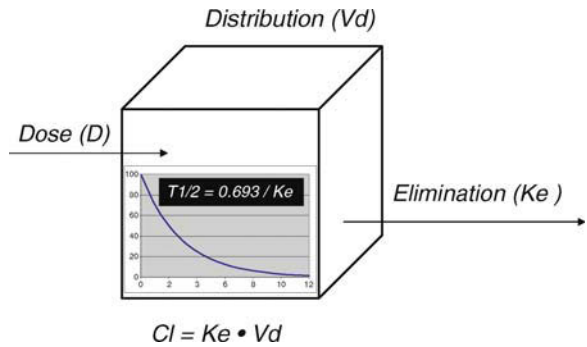
$$Cl = 0.693 * V_d / T_{1/2}$$

The half-life can be read off from the time interval ($t_{0.50} = T_{1/2}$) or ($t_{0.10} = 3.3 * T_{1/2}$) or ($t_{0.05} = 4.32 * T_{1/2}$) or the interval ($t_2 - t_1$) between any concentration-fraction (C_2 / C_1).

$$T_{1/2} = 0.693 * (t_2 - t_1) / \ln(C_1 / C_2)$$

Continuous Infusion, Zero Order, and Michaelis–Menten Kinetics

If the drug is administered by a constant infusion rate (IR), the curve follows an unsteady function with zero-order kinetics ($dC/dt = \text{const.}$) before the infusion is stopped ($t < T_{infus}$) and first-order kinetics after cessation of infusion. Zero-order kinetics frequently can also be observed with drug absorption where ($K_{0abs} = IR$) and ($T_{abs} = T_{infus}$) hold true.



Pharmacokinetics. Figure 1 Main pharmacokinetic processes and parameters: Half-life ($T_{1/2}$), volume (V_d), elimination rate constant (K_e), and clearance (Cl).

$$C = (IR / Cl) * (1 - \exp(-0.693 * t / T_{1/2}))$$

$$C_{ss} = IR / Cl$$

$$C = (IR / Cl) * (1 - \exp(-0.693 * T_{infus} / T_{1/2})) * \exp(-0.693 * (t - T_{infus} / T_{1/2}))$$

Log-concave kinetics can be due to saturable Michaelis–Menten elimination depending on the maximal metabolism capacity (V_{max}) and the Michaelis constant (K_m).

$$-dC/dt = V_{max} * C / (K_m + C)$$

$$T_{1/2} = 0.693 * (K_m + C) / V_{max}$$

With high concentration values ($C \gg K_m$), the metabolism capacity is saturated and zero-order kinetics result ($-dC/dt = V_{max}$).

$$C = -V_{max} * t$$

But with low concentration values ($C \ll K_m$), first-order kinetics result ($-dC/dt = (V_{max} / K_m) * C$).

$$C = C_0 * \exp(-(V_{max} / K_m) * t)$$

With the Michaelis–Menten equation, there is no integrated solution for the concentration, but only for the time

$$t = (C_{max} - C) / V_{max} + (K_m / V_{max}) * \ln(C_{max} / C)$$

However, saturable kinetics can also be described by a 1-exp function. This function indeed has an integrated solution for the concentration.

$$-dC/dt = V_{max} * (1 - \exp(-C / K_m))$$

$$C = K_m * \ln[1 - (1 - \exp(C_{max} / K_m))] * \exp(-V_{max} * t / K_m)$$

Bi-exponential Kinetics

After intravenous drug administration, a log-convex concentration decline points to a multi-exponential function. For the most frequent case, a bi-exponential equation with the inter-compartment rate constants (k_{12}) and (k_{21}) can be fitted.

$$C = Ca * \exp(-\alpha * t) + Cb * \exp(-\beta * t)$$

$$Co = Ca + Cb$$

$$AUC = Ca/\alpha + Cb/\beta$$

$$T1/2\alpha = 0.693/\alpha$$

$$T1/2\beta = 0.693/\beta$$

$$k21 = (Ca * \beta + Cb * \alpha) / (Ca + Cb)$$

$$k10 = \alpha * \beta / k21$$

$$k12 = \alpha + \beta - k21 - k10$$

The concentration (C_{tiss}) in the peripheral volume ($V2$) or tissue compartment follows also a bi-exponential function.

$$C_{tiss} = (Co * k12 / (\alpha - \beta)) * (\exp(-\beta * t) - \exp(-\alpha * t))$$

The concentration of a metabolite (Cm), too, follows such a bi-exponential kinetics depending on the metabolite formation rate constant ($Kmet$).

$$Cm = Cm0 * (\exp(-Kmet * t)) - \exp(-Ke * t)$$

With bi-exponential kinetics it can be difficult to select the appropriate volume term, since several volume parameters can be derived.

$$V_{ss} = V1 + V2$$

$$V1 = D/Co$$

$$V2 = V1 * (k12/k21)$$

$$Vd = V1 * k10/\beta$$

$$Vd = D / (\beta * AUC)$$

$$V_{extrap} = D/Cb$$

It is the advantage of the clearance parameter that only one clearance term is obtained.

$$Cl = k10 * V1$$

$$Cl = \beta * Vd$$

One solution to the volume problem was proposed using moment analysis. The steady-state volume of distribution (V_{ss}) can be derived from the area under the curve (AUC) and the area under the first moment curve (AUMC).

$$V_{ss} = D * AUMC / (AUC^2) = Cl * MRT$$

$$MRT = AUMC / AUC$$

The mean residence time (MRT) gives one parameter for the multi-exponential elimination kinetics with more than one half-life.

$$AUC = \sum_{i=1}^n (C_{i-1} + C_i) * (t_i - t_{i-1}) / 2 + C_n / \lambda_{bdaz}$$

$$AUC = \sum_{i=1}^n Ca / \lambda_{bdaa}$$

$$AUMC = \sum_{i=1}^n (t_i - t_{i-1}) / 2 + t_n * C_n / \lambda_{bdaz} + C_n / \lambda_{bdaz}^2$$

$$AUMC = \sum_{i=1}^n Ca / \lambda_{bdaa}^2$$

The dominant half-life must represent 90% of the AUC ($0.90 * AUC = 1.44 * Co * T1/2_{dom}$), and it corresponds to the MRT estimate where ($1/\ln 2 = 1.44$).

$$T1/2_{dom} = 0.693 * MRT$$

Oral Dose and Bioavailability

After oral dosing, the bi-exponential Bateman function holds true with the absorption rate constant (Ka).

$$C = Co * (Ka / (Ka - ke)) * (\exp(-Ke * t) - \exp(-Ka * t))$$

$$Co = F * D / Vd$$

$$t_{max} = \ln(Ka/Ke) / (Ka - Ke)$$

$$C_{max} = Co * \exp(-Ke * t_{max})$$

The elimination half-life after oral dosing is longer than after intravenous dosing ($T1/2_{po} > T1/2_{iv}$) when the so-called “flip-flop” condition applies ($Ka < Ke$).

$$T1/2_{iv} = 0.693/Ke$$

$$T1/2_{po} = 0.693/Ka$$

According to the law of corresponding areas (FH Dost), the area derived from the integrated Bateman function ($AUC_{po} = AUC_{iv}$) is the same as the area after intravenous dosing if the **bioavailability** (F) is complete.

$$AUC_{po} = Co * (Ka / (Ka - Ke)) * (1/Ke - 1/Ka)$$

$$AUC_{po} = Co/Ke$$

The systemically available fraction or bioavailability (F) can be defined by the fraction of the area after oral and intravenous dosing, respectively.

$$F = AUC_{po} / AUC_{iv}$$

The apparent clearance (Cl/F) and the apparent volume (Vd/F) estimates are related to the bioavailability after oral dosing.

$$Cl/F = D / AUC_{po}$$

$$Vd/F = D / (\beta * AUC_{po})$$

A special case occurs with the entero-hepatic recycling of a drug that can be described by using the sum of two Bateman-like functions.

$$C = Cal * (\exp(-Kel * t) - \exp(-Kal * t)) + Ca2 * (\exp(-Ke2 * t) - \exp(-Ka2 * t))$$

A special case for reduced bioavailability results from first-pass extraction that sometimes might be subjected to saturable Michaelis–Menten absorption kinetics. The lower the hepatic drug clearance is (Cl_{hep}) in relation to liver blood flow (Ql), or the faster the drug absorption rate constant (Ka), and the higher the dose (D) are, the more bioavailable is the drug (F).

$$F = 1 - Cl_{hep}/Ql$$

$$F = 1 - ((Vmax * Vd) / (Ka * D)) * \ln(1 + (Ka * D) / (Km * Ql))$$

Plasma Binding

The free plasma fraction (fp) determines the relation between free, bound (►plasma binding PB%), and total concentration.

$$\begin{aligned} PB\% &= 1 - fp \\ fc &= C_{free}/C \\ C &= C_{free} + C_{bound} \\ fp \uparrow &= C_{free}/(C_{free} + C_{bound} \downarrow) \\ fp \uparrow &= C_{free}/C \downarrow \\ C_{free} &= \text{const.} \end{aligned}$$

The total clearance increases in proportion to the free plasma fraction while free plasma clearance remains constant.

$$\begin{aligned} fp \uparrow * C \downarrow &= C_{free} = \text{const.} \\ fp * AUC &= \text{const.} \\ Cl_{free} &= F * D / AUC_{free} \\ AUC_{free} &= \text{const.} \\ CL \uparrow &= fp \uparrow * Cl_{free} \\ Cl_{free} &= \text{const.} \end{aligned}$$

The apparent volume of distribution (Vd) slightly increases depending on plasma volume (Vp), tissue volume (Vt), and free tissue fraction (ft) whereas the half-life slightly decreases with significantly increasing free plasma fraction.

$$\begin{aligned} Vd \uparrow &= Vp + Vt * fp \uparrow \uparrow / ft \\ T1/2 \downarrow &= (0.693 / Cl_{free}) * (Vd \uparrow / fp \uparrow \uparrow) \end{aligned}$$

Since the binding capacity (B_{max}) is saturable and depending on the affinity constant (K_{aff}), the free plasma fraction (fp) increases with increasing total and thus also bound concentrations.

$$\begin{aligned} C_{bound}/C_{free} &= B_{max} - K_{aff} * C_{bound} \\ fp &= 1 / (1 + B_{max} - K_{aff} * C_{bound}) \end{aligned}$$

Fortunately, changes in drug binding are expected to have no clinical relevance except for TDM where a new target concentration (C_{target}) must be explicitly stated – usually lower than with normal ►plasma binding.

$$C_{target} \downarrow = C_{free} + C_{bound} \downarrow .$$

Renal Impairment

The kidney function confers high impact on drug kinetics when a high fraction ($fren$) of the drug dose (D) is eliminated in urine (Aurine). The renal fraction ($fren$) can be underestimated when bioavailability (F) is neglected. The renal clearance, however, can be derived from the amount eliminated by the renal route (Aurine) independent from bioavailability (F).

$$fren * F = \text{Aurine} / D_{oral}$$

$$Cl_{ren} = \text{Aurine} / AUC$$

$$fren * F = Cl_{ren} / Cl$$

Total drug clearance is the sum of nonrenal clearance and renal clearance (Cl_{ren}). According to the MDRD-2 formula, the estimated GFR (eGFR) is a function of serum creatinine (SCr in mg/dl) and age (Age in years). It has the unit ml/min per 1.73 m^2 .

$$Cl = Cl_{nonren} + Cl_{ren}$$

$$Cl_{ren} = A * eGFR$$

$$eGFR = 186 \times [SCr]^{-1} \times [Age]^{-0.203}$$

$$Cl = Cl_{nonren} + A * eGFR$$

The renal clearance can be underestimated in the case of renal drug metabolism. The total drug clearance depends on bioavailability. Therefore, the most reliable estimate for the fraction eliminated by the renal route ($fren$) is given by the normal clearance (Cl_{norm}) and drug clearance in case of acute and/ or chronic renal failure (Cl_{fail}), or from half-lives ($T1/2_{norm}$) and ($T1/2_{fail}$).

$$fren = 1 - Cl_{fail} / Cl_{norm}$$

$$fren = 1 - T1/2_{norm} / T1/2_{fail}$$

The interpolation of individual clearance (Cl), volume (Vd), plasma binding ($PB\%$), and half-life ($T1/2$) for any stage of renal impairment is obtained from the normal clearance (Cl_{norm}), volume (Vd_{norm}), plasma binding estimate ($PB\%_{norm}$), or half-life ($T1/2_{norm}$), and the parameters for kidney failure (X_{fail}). The individual renal function is estimated by the GFR (eGFR = MDRD-2) where (eGFR_{fail} = 0).

$$Cl = Cl_{fail} + A * eGFR$$

$$Vd = Vd_{norm} + / - B * (eGFR - eGFR_{norm})$$

$$PB\% = PB\%_{norm} + / - G * (eGFR - eGFR_{norm})$$

$$T1/2 = T1/2_{norm} / (1 - fren + fren * eGFR / GFR_{norm})$$

With

$$A = (Cl_{norm} - Cl_{fail}) / eGFR_{norm}$$

$$B = - / + (Vd_{fail} - Vd_{norm}) / eGFR_{norm}$$

$$G = - / + (PB\%_{fail} - PB\%_{norm}) / eGFR_{norm}$$

With bi-exponential kinetics, it should be sufficient to linearly correlate beta with eGFR.

$$\text{beta} = \text{beta}_{fail} + (\text{beta}_{norm} - \text{beta}_{fail}) *$$

$$eGFR / eGFR_{norm}$$

The volume will decrease when renal impairment is associated with a decrease in the elimination rate beta ($\text{beta}_{norm} \Rightarrow \text{beta}_{fail}$). On the other side, the volume will increase when the free plasma fraction (fp) increases in renal impairment where $fp = 1 - PB\%$. The volume decreases, when the free tissue fraction (ft) increases in renal impairment.

$$Vd = V1 * (1 + k12 / (k21 - \beta))$$

$$Vd = V1 + V2 * fp / ft$$

Hemofiltration and Hemodialysis

The effect of ►hemofiltration on drug elimination can be estimated from serum creatinine (SCr), age, and the MDRD-2 formula to predict the combined effect of filtration rate (eGFR = GFR_{residual} + HFR) on drug clearance and drug half-life during hemofiltration.

$$T1/2 = T1/2_{\text{norm}} / (1 - \text{fren} + \text{fren} * \text{eGFR} / \text{GFR}_{\text{norm}})$$

$$Cl = Cl_{\text{fail}} + A * \text{eGFR}$$

The effect of ►hemodialysis can be derived from the removed fraction (FR) that is the relative amount eliminated from the body during the time (*t*_{HD}) of one dialysis session. This fraction can be derived from the half-life on dialysis (*T*_{1/2on}) or from the area under the curve (AUC) on and off dialysis.

$$FR = 1 - \exp(-0.693 * t_{\text{HD}} / T1/2_{\text{on}})$$

$$FR = (AUC_{\text{off}} - AUC_{\text{on}}) / AUC_{\text{off}}$$

The total fraction (FR) eliminated must be distinguished from the specific fraction (FR_{fract}) that is eliminated by the dialysis alone.

$$FR_{\text{fract}} = \exp(-0.693 * t_{\text{HD}} / T1/2_{\text{off}})$$

$$- \exp(-0.693 * t_{\text{HD}} / T1/2_{\text{on}})$$

After dialysis, often a rebound is seen in concentrations since elimination from plasma is faster than drug flux from tissue to plasma (*C*_{rebound} = *C*_{tissue} - *C*). The concentration in plasma follows a bi-exponential kinetics during hemodialysis whereas the concentration in tissue follows mono-exponential kinetics.

$$C = C_0 * (\exp(-\alpha t_{\text{HD}}) + \exp(-\beta t_{\text{HD}}))$$

$$C_{\text{tissue}} = C_0 * \exp(-\beta t_{\text{HD}})$$

$$C_{\text{rebound}} = -C_0 * \exp(-\alpha t_{\text{HD}})$$

For a prolonged dialysis duration (*t* = 3/α = 4.32 * *T*_{1/2α}), there will be no longer a rebound after termination of dialysis.

Accumulation Kinetics and Multiple Dosing

If a drug is given repetitively with a constant dose (*D*) and a constant administration interval (*Tau*) accumulation occurs until a steady-state concentration (*C*_{ss}) is obtained after 4.32 times the elimination half-life (*t*_{ss} = 4.32 * *T*_{1/2}).

$$C_{\text{ss}} = F * D / (Cl * \text{Tau})$$

In the steady state, the peak concentration and the trough concentration can be distinguished.

$$C_{\text{peak}} = C_0 / (1 - \exp(-0.693 * \text{Tau} / T1/2))$$

$$C_{\text{trough}} = C_0 / (\exp(0.693 * \text{Tau} / T1/2) - 1)$$

The calculated peak concentration is not always the measured concentration. For bi-exponential kinetics the calculated peak concentration is less, but after oral dosing, the calculated peak concentration is higher than the highest measurable plasma concentration. The peak concentration provides a target for the loading dose to start with (*D*_{load}).

$$D_{\text{load}} = C_{\text{peak}} * Vd$$

For bi-exponential accumulation kinetics the contribution of the fast alpha phase (*R*_α = 1/(1 - exp(-α * *Tau*))) decreases (*R*_α * *C*_a << *R*_β * *C*_b) as compared with the contribution of the slow beta-phase (*R*_β = 1/(1 - exp(-β * *Tau*))).

Pharmacodynamics

The drug dose (*D*) produces a concentration (*C*) and the concentration produces an effect (*E*).

$$D \Rightarrow PK \Rightarrow C \Rightarrow PD \Rightarrow E$$

The most general equation for the correlation between effect (*E*) and concentration (*C*) is given by the sigmoid *E*_{max} model where the concentration (*C*_{E50}) produces the half-maximum effect and the Hill coefficient (*H*) specifies the sigmoidicity (Fig. 2).

$$E = E_{\text{max}} / (1 + (C_{\text{E50}} / C)^H)$$

If the Hill coefficient is less than one (*H* < 1), the maximum effect will never be obtained with increasing concentrations (*E* < *E*_{max}). If the Hill coefficient is more than ten (*H* > 10), an on-off phenomenon can be described at *C*_{E50}. The effect can be assumed to be marginal and less than 5% of *E*_{max} for the threshold concentration (*C*_{E05}) but almost 95% of *E*_{max} for the ceiling concentration (*C*_{E95}).

$$C_{\text{E05}} = C_{\text{E50}} * 0.053^{(1/H)}$$

$$C_{\text{E95}} = C_{\text{E50}} * 0.053^{(-1/H)}$$

Established standard dosage produces the target concentration (*C*_{target}) between normal peak and trough levels.

$$C_{\text{peak}} = C_{\text{trough}} * \exp(0.693 * \text{Tau} / T1/2)$$

$$C_{\text{trough}} \approx C_{\text{E05}}$$

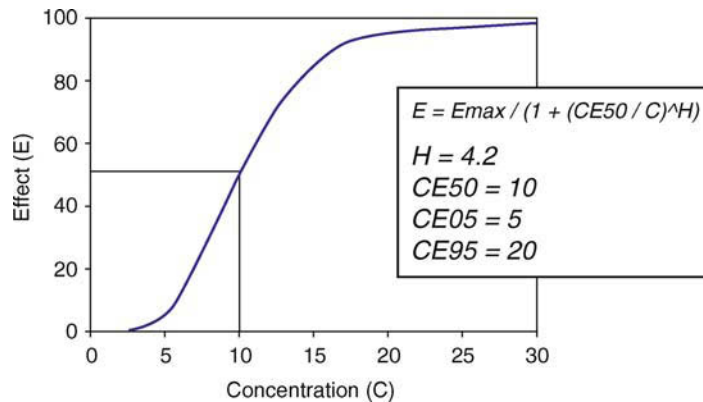
$$C_{\text{peak}} \approx C_{\text{E95}}$$

The time of effect duration (TED) is a function of the elimination half-life. Two special cases are most important, the 50% effect bisection time (TED₅₀) and the duration of 90% of the effect (TED₉₀).

$$\text{TED}_{50} = T1/2 * (1.44/H) * \ln(2 + (C_{\text{peak}} / C_{\text{E50}})^H)$$

$$\text{TED}_{90} = T1/2 * (1.44/H) * \ln(10 + 9 * (C_{\text{peak}} / C_{\text{E50}})^H)$$

For single dose monoexponential kinetics and direct effect conditions, the area under the effect time curve (AUEC) can be derived by integration of the Hill equation.



Pharmacokinetics. Figure 2 Sigmoid $E_{\max}$ model of pharmacodynamics with Hill coefficient (H), concentration producing half-maximum effect (CE_{50}), threshold concentration (CE_{05}), and ceiling concentration (CE_{95}).

$$AUEC = 1.44 * T_{1/2} * (E_{\max}/H) * \ln(1 + (C_0/CE_{50})^H)$$

For irreversible effects no ceiling concentration is assumed; the incremental increase in concentrations and the absolute height of the peak might be more impacting.

Pharmacological Relevance

Luzius Dettli was not only the first who described the linear function for the dependence of drug elimination on glomerular filtration rate. He was also the first who proposed the fundamental dose adjustment recommendation, the proportional dose reduction rule. Two alternatives are given to either reduce the single dose (D) or extend the interval (τ).

$$D/\tau = (D/\tau)_{\text{norm}} * Cl/Cl_{\text{norm}}$$

$$D/\tau = (D/\tau)_{\text{norm}} * T_{1/2\text{norm}}/T_{1/2}$$

If $(\tau)_{\text{norm}} = \text{const.}$

$$D = D_{\text{norm}} * Cl/Cl_{\text{norm}}$$

$$D = D_{\text{norm}} * T_{1/2\text{norm}}/T_{1/2}$$

The administration interval could be selected based on standard peak and trough concentrations or from effect duration time (TED_{50} , TED_{90}). If the dose is reduced, a loading dose ($D_{\text{load}} = C_{\text{peak}} * V_d = D_{\text{norm}}$) must be administered to obtain an immediate effect. If $D_{\text{norm}} = \text{const.}$

$$\tau = (\tau)_{\text{norm}} * Cl_{\text{norm}}/Cl$$

$$\tau = (\tau)_{\text{norm}} * T_{1/2}/T_{1/2\text{norm}}$$

A second, completely different dose adjustment rule exists. Calvin Kunin proposed to start with the standard dose and to administer one half of the standard dose after one individual half-life. The half-dosage rule of Kunin should only be applied if the individual half-life is longer than the standard administration interval.

$$D_{\text{load}} = D_{\text{norm}}$$

$$D/\tau = 1/2 * D_{\text{load}}/T_{1/2}$$

$$D = 1/2 * D_{\text{load}}$$

$$\tau = T_{1/2}$$

$$C_{\text{peak}} = \text{const.}$$

$$C_{\text{trough}} > (C_{\text{trough}})_{\text{norm}}$$

The Dettli rule might result in underdosage because of too infrequent peak levels; the Kunin rule might result in overdosage because of toxic trough levels. The third, the target concentration approach was proposed by Nikolas Holford.

$$D/\tau = C_{\text{target}} * Cl$$

$$C_{\text{target}} = CE_{50} * E/(E_{\max} - E)$$

The effect area should remain constant ($AUEC = \text{const.}$). Thus the target concentration (C_{target}) might be derived from the normal peak concentration (C_{peak}).

$$C_{\text{target}} = CE_{50} * [(1 + (C_{\text{peak}}/CE_{50})^H)^H * (T_{1/2\text{norm}}/T_{1/2}) - 1]^{(1/H)}$$

When the half-life is prolonged, the target concentration will decrease ($C_{\text{target}} < C_{\text{peak}}$).

Supplementary Dose after Hemodialysis

During the time on dialysis (t_{HD}) a fraction (FR) of the amount in the body is eliminated. The required amount in the body often corresponds to the loading dose (D_{load}) or to $(D_{\text{load}} - D_{\text{fail}})$. This fraction must be replaced by a supplementary dose (D_{suppl}) to maintain therapeutic drug levels for the time interval between two dialysis sessions.

$$D_{\text{suppl}} = FR * (D_{\text{load}} - D_{\text{fail}})$$

The dose after dialysis (D_{hd}) must replace the maintenance dose adjusted to renal failure (D_{fail}) and the

supplementary dose (D_{suppl}) that both are functions of the loading dose. According to the Kunin rule one obtains for the most general conditions:

$$D_{\text{hd}} = D_{\text{fail}} + D_{\text{suppl}}$$

$$D_{\text{fail}} = \frac{1}{2} * D_{\text{load}}$$

$$D_{\text{suppl}} = FR * D_{\text{load}}$$

$$D_{\text{hd}} = (1/2 + FR) * D_{\text{load}}$$

► Trace Amines

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diseases caused by misfolded proteins, like cystic fibrosis (*CFTR* gene mutations), retinitis pigmentosa (*RHO* gene mutations), liver cirrhosis due to α 1-antitrypsin deficiency (*SERPINA1* gene mutations) and inherited nephrogenic diabetes insipidus (*AVPR2* and *AQP2* gene mutations).

- Chaperone
- Protein Trafficking and Quality Control
- Vasopressin/Oxytocin
- CFTR

Pharmacophore Modelling

Computer-assisted approach which aims at deriving a detailed model of the ligand-binding pocket(s) of a receptor or enzyme from biochemical and pharmacological data obtained in pharmacological experiments such as ligand-binding assays and receptor mutagenesis studies. According to IUPAC a pharmacophore is considered an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or inhibit) its biological response.

Pharmacological Barriers

Physiological barriers limiting the passage of pharmacological compounds within the body.

► MDR-ABC Transporters

Pharmacological Chaperone

Pharmacological chaperones are substances that selectively facilitate misfolded proteins to fold and thereby allowing them to gain certain wild-type characteristics, like promoting their cell surface expression and/or rescuing their functional activity. They can be ligands, substrates or inhibitors of unstable receptors, transmembrane channels or enzymes. These substances are promising drugs for a specific therapeutic approach for

Pharmacodynamic Tolerance

Pharmacodynamic tolerance develops in response to continued application of drugs, by mechanisms that include reversible cellular adaptation processes, such as receptor desensitization, internalization and downregulation as well as changes in the activity and levels of other components of the receptor's signal transduction pathways.

► Tolerance and Desensitization

Phase I and Phase II Metabolism

The metabolism of foreign compounds (xenobiotics) often takes place in two consecutive reaction, classically referred to as phases one and two. Phase I is a

functionalization of the lipophilic compound that often is the prerequisite for further conjugation reactions, also termed phase II. The conjugated product is usually sufficiently water soluble to be excretable into the urine. The most important biotransformations of Phase I are aromatic and aliphatic hydroxylations catalyzed by cytochromes P450. Other phase I enzymes are for example epoxide hydrolases or carboxylesterases. Typical phase II enzymes are UDP-glucuronosyltransferases (UGT), sulfotransferases (SULT), N-acetyltransferases (NAT) and methyltransferases (e.g. thiopurin S-methyltransferase or TPMT).

- P450 Mono-oxygenase System
- Pharmacokinetics

Phase Shift

An alteration in the endogenous circadian clock, most often elicited by a change in the light/dark cycle, for example when travelling between time zones.

- Sleep

Phenothiazines

- Antipsychotic Drugs

Phenotype

Phenotype is the actual appearance of an organism or, more specifically in the research context, of a trait of interest. It may be a binary trait, i.e., the presence/absence of a disease, for example essential hypertension, or a quantitative trait, such as blood pressure level.

- Pharmacogenomics

Phenylalkylamines

A family of compounds blocking dihydropyridine-sensitive HVA calcium channels.

- Voltage-dependent Ca^{2+} Channels

PHHI

Persistent Hyperinsulinemic Hypoglycemia of Infancy.

- Familial Persistent Hyperinsulinemic Hypoglycemia of Infancy

PHLPP

A phosphatase that dephosphorylates a conserved carboxyl-terminal site on PKC and Akt, thus inactivating these kinases and terminating their signaling pathways.

- Protein Kinase C

Phorbol Esters

Natural products from the Euphorbiaceae family of plants that mimic the effects of diacylglycerol by binding the C1 domain of proteins such as PKC.

- Protein Kinase C

Phosphatase

A phosphatase is an enzyme that hydrolyzes phosphomonoesterases, a reaction yielding free phosphate and alcohol. Substrates for phosphatases include both

phospholipids and proteins. A wide variety of protein phosphatases exist and are generally classified according to their substrate specificities. For example, protein tyrosine phosphatases dephosphorylate phosphotyrosine residues while dual specificity phosphatases dephosphorylate both phosphothreonine and phosphotyrosine residues.

► Protein Phosphatases

Phosphate Transferring Enzyme Inhibitors

► Protein Kinase Inhibitors

Phosphatidic Acid

Phosphatidic acid is glycerol esterified at the sn-1 and sn-2 positions to two fatty acids and at the sn-3 position to phosphoric acid. It is a product of phospholipase D action that is also an intermediate in the biosynthesis of phosphatidylserine and phosphatidylinositol.

► Phospholipases

Phosphatidylinositide Kinases

► Phospholipid Kinases

Phosphatidylinositol

Phosphatidylinositol (abbreviated PtdIns, or PI) is a minor class of phospholipids composed of glycerol, fatty acids and inositol. PIs are found in the cytosolic side of eukaryotic cell membranes. They are substrates for a large number of enzymes which are involved in cell signalling.

► Phospholipid

Phosphatidylinositol 4,5-bisphosphate

Phosphatidylinositol 4,5-bisphosphate is a derivative of phosphatidylinositol in which the inositol ring is phosphorylated at positions 4 and 5.

► Phospholipases

Phosphatidylinositol 3-kinase

Family of enzymes phosphorylating phosphatidylinositol (PtdIns), PtdIns(4)phosphate, and PtdIns(4,5)phosphate in the 3-position. The PtdIns(3)phospholipids are second messengers in processes like cell growth, cytoskeletal rearrangement, and vesicular transport. PI 3-kinases are heterodimers composed of a catalytic and a regulatory subunit. The enzymes are activated by insulin, many growth factors, and by a variety of cytokines. Their activity can be inhibited by wortmannin and LY294002.

► Insulin Receptor

► Phospholipid Kinases

Phosphatidylinositol Kinases

► Phospholipid Kinases

Phosphatidylinositol Phosphate

Phosphatidylinositol phosphates (PIPs) are phosphorylated derivatives of PI (phosphatidylinositol). PIPs that have been detected in cells include PI-3-P, PI-4-P (PIP), PI-5-P, PI-3,4-P₂, PI-4,5-P₂(PIP₂), PI-3,5-P₂, and PI-3,4,5-P₃(PIP₃). PIP and PIP₂ are the most abundant forms (~60%).

► ATP-dependent K⁺ Channels

► Phospholipases

Phosphatidylinositol

Phosphatidylinositol is a phospholipid containing the inositol sugar head group.

- Phospholipases
- Protein Phosphatases

Phosphodiesterases

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Synonyms

Cyclic nucleotide phosphodiesterases; PDEs

Definition

Cyclic nucleotide phosphodiesterases (PDEs) are a class of enzymes that catalyze the hydrolysis of 3',5'-cyclic guanosine monophosphate (cGMP) or 3',5'-cyclic adenosine monophosphate (cAMP) to 5'-guanosine monophosphate (GMP) or 5'-adenosine monophosphate (AMP), respectively.

Basic Characteristics

The cyclic nucleotide PDEs are a superfamily of enzymes that hydrolyze cGMP and cAMP to their respective 5'-monophosphates. Cyclic nucleotide signaling is important in a large number of processes including proliferation, ►chemotaxis, muscle contraction, and relaxation, energy metabolism, and inflammation. Therefore, together with the cyclases (adenylyl and guanylyl cyclase), the PDE family is crucial for survival and for maintaining quality of life in eukaryotic organisms. Currently, there are 11 known PDE gene families, all of which have unique characteristics in terms of substrate specificity, expression patterns, kinetics, and regulatory properties (Table 1). The current nomenclature for a PDE contains, in order, two letters to indicate species, a number indicating gene family, a letter to represent an individual gene, and a number to signify splice or start site variant. For example, MMPDE9A1 represents the mouse PDE9 gene family, gene A, splice variant 1. In general, all PDEs share the same structural organization. Each protein has an N-terminal motif that is used to regulate

the protein's activity or localization, and a C-terminal catalytic domain, followed by a short tail. The catalytic domains of all Class 1 PDEs share approximately 35% sequence identity, including a conserved signature sequence, H-D-X₂-H-X₄-N.

Substrate Specificity

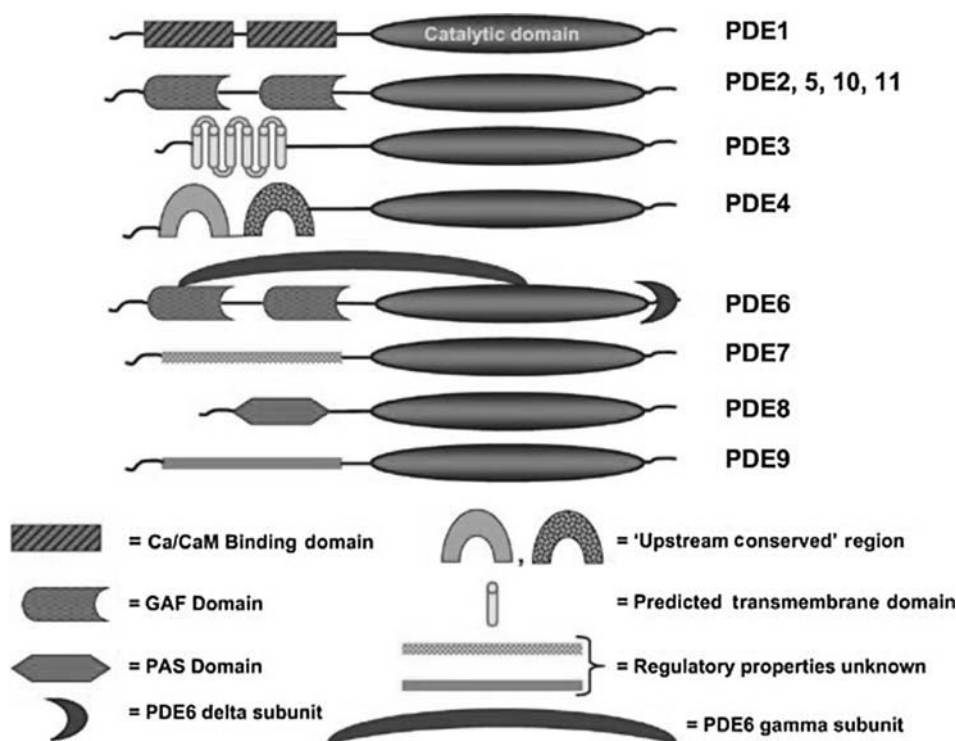
The substrate specificity of the PDEs range from dual specificity enzymes (PDE1, 2, 10, and 11) to those that preferentially hydrolyze either cGMP (PDE5, 6, and 9) or cAMP (PDE3, 4, 7, and 8). In fact, the relative substrate specificities can vary between members of a gene family. For example, within the PDE1 gene family, PDE1A has a *K_m* for cGMP that is much lower than that for cAMP, while PDE1C has roughly equal affinity for both nucleotides. In addition, the activity of a PDE toward one cyclic nucleotide can vary depending on the concentration of the other cyclic nucleotide. For instance, PDE2 will hydrolyze cAMP and cGMP with relatively similar *K_m* values. However, the presence of a small amount of cGMP (through allosteric binding sites) stimulates the activity of PDE2 towards cAMP. There are also PDEs for which one cyclic nucleotide acts as a competitive inhibitor for the other. For example, cAMP is a competitive inhibitor of cGMP hydrolysis by PDE10, and cGMP is a potent inhibitor of cAMP hydrolysis by PDE3s. Thus, with a wide variety of specificities and affinities for the different nucleotides, the PDEs are a group of enzymes that are well adapted to fine tune the cyclic nucleotide pools within the cell.

Regulatory Domains

All of the PDEs possess a significant amount of sequence that is N-terminal to the catalytic domain. In fact, the N-terminus can be larger than the catalytic domain itself. Within the N-terminus there are domains used to regulate the activity of the catalytic site (Fig. 1). The PDE1 family members have two Ca²⁺/calmodulin-binding domains. Binding of Ca²⁺/calmodulin to these domains stimulates the activity of the PDE1s several fold. PDE2, PDE5, PDE6, PDE10, and PDE11, all have allosteric cGMP-binding or cAMP-binding GAF domains (named after cyclic GMP-binding PDE, ►adenylyl cyclase, FhlA) in their N-termini. Binding of cGMP or cAMP to these domains have various effects on PDEs. For PDE2, binding of cGMP to its GAF domain results in stimulation of its cAMP hydrolyzing activity. Binding of cGMP to PDE5 increases its susceptibility to phosphorylation, and also has a direct effect on activity. PDE10 and PDE11 have unusual GAF domains. The affinity of PDE10 for cGMP is low, with a reported *K_d* of greater than 10 μM. It has a much higher affinity for cAMP. In the PDE11 family, there are reported splice variants with full length and truncated GAF domains. It is unclear, what role these truncated forms might play, if any, in PDE11 function. The N-terminus of PDE8 has another small molecule

Phosphodiesterases. Table 1 Characteristics of the individual Class 1 cyclic nucleotide PDE families

PDE Family	Genes	Probable splice variants	Regulatory domains/role	Phosphorylation	Substrate (s)	Commonly used inhibitors
PDE1	1A, 1B, 1C	9	CaM/activation	PKA	cGMP, cAMP	KS-505
PDE2	2A	3	GAF/activation	Unknown	cAMP, cGMP	EHNA
PDE3	3A, 3B	1 for 3A; 2 for 3B	Transmembrane domains/membrane targeting	PKB, PKA	cAMP > cGMP	Milrinone
PDE4	4A, 4B, 4C, 4D	>20	UCR1, UCR2/unclear	ERK, PKA	cAMP	Rolipram
PDE5	5A	3	GAF/activation	PKA, PKG	cGMP	Sildenafil, Vardenafil, Tadalafil,
PDE6	6A, 6B, 6C	1 each	GAF/activation	PKC, PKA	cGMP	Sildenafil, Dipyridamole, Zaprinast
PDE7	7A, 7B	6	Unknown	Unknown	cAMP	None identified
PDE8	8A, 8B	6	PAS/unknown	Unknown	cAMP	None identified
PDE9	9A	4	Unknown	Unknown	cGMP	None identified
PDE10	10A	2	GAF/activation?	Unknown	cAMP, cGMP	None identified
PDE11	11A	4	GAF/unknown	Unknown	cAMP, cGMP	None identified

**Phosphodiesterases. Figure 1** Domain organization of the phosphodiesterase family.

binding domain, the PAS domain. PAS domains are found in a number of eukaryotic systems and are found within proteins that are involved in sensing their environment such as oxygen, energy, or light levels.

They also bind small molecules and can be involved in protein-protein interactions. PAS domains in other proteins commonly function either as protein interaction sites or small molecule binding domains. Occasionally,

they serve both functions. However, it is not known for PDE8 whether the PAS domain has either of these functions. It will be of great interest if a small molecule regulatory ligand that binds to the PAS domain can be identified.

PDEs are also regulated by targeting and phosphorylation. Some of the PDE3 family members have six putative transmembrane domains in their N-terminal regions, which is the likely reason much PDE3 activity is largely membrane associated. Some PDEs, such as PDE2A2 and PDE9A1, have putative N-myristoylation sites which presumably target these proteins to the membrane. While this has not been demonstrated for PDE9, a membrane associated form of PDE2 activity has been shown. PDE6, the PDE involved in retinal [phototransduction](#), is prenylated and tightly associated with the disc membranes of the photoreceptor cells. Lipid modification, however, is not the only mechanism for localizing PDEs. Examples of proteins that associate with PDEs and likely target them to cellular compartments are emerging. Recently, RACK1, a [scaffolding protein](#) that associates with PKC and Src, was also shown to bind PDE4D5. PDE4D also associates with a centrosomal protein, myomegalin, and a PKA anchoring protein, mAKAP. All of these associations with PDE4D are likely to be targeting this PDE to specific cAMP pools in the cell. Interactions of PDE4 family members with XAP2 immunophilins, src like tyrosine kinases, β -arrestins, DISC1, and several A-kinase anchoring proteins (AKAPs) have all been reported. Many of these are known to have functional consequences. Undoubtedly, more examples of this type of targeting will be discovered.

Compartmentalization of PDEs

Compartmentalization of cyclic nucleotide signaling within the cell is a notion that has been around for some 20 years, yet has been difficult to demonstrate for the PDEs. However, some examples of compartmentalization of PDEs in cells have been shown. The best studied example is in the photoreceptor, where PDE6 is concentrated on the membrane disks along with the other players in the phototransduction cascade. There, PDE6 is activated in response to light and hydrolyzes local cGMP, and resident cGMP-gated cation channels close, hyperpolarizing the cell. In kidney mesangial cells, the production of [superoxide](#) and mitogenesis are both stimulated through the elevation of cAMP. The production of superoxide is rolipram sensitive, indicating a role for PDE4 in this process. Mitogenesis is cilostamide sensitive, implying a role for PDE3. Superoxide generation is not effected by cilostamide and mitogenesis is insensitive to rolipram. Thus, while both of these processes are cAMP-dependent, different cAMP pools and PDEs are involved in each. Another example of compartmentalization of PDE activities in the cell exists in the olfactory epithelium, where

PDE1C2 and PDE4A are expressed. PDE1C2 is found in the cilia of the epithelium, where it colocalizes with adenylyl cyclase. PDE4A is found throughout the epithelial layer, but not in cilia. Therefore, as in the kidney mesangial cells, different PDEs must be working on different cyclic nucleotide pools. More recently, substantial data has been developed for compartmentation of cAMP and PDEs in cardiac myocytes.

Drugs

Currently, Viagra (Pfizer, Inc.) and its more recent competitors Levitra[®] and Cialis, are the best examples of specific PDE inhibitors put to clinical use. Stimulation of the smooth muscle cells of the corpus callosum by nitric oxide results in the elevation of cGMP. This increase in cGMP levels results in relaxation of the vascular smooth muscle and engorgement of the penis. Viagra, through inhibition of PDE5 in the smooth muscle cells, potentiates this effect. Because it is highly selective for PDE5, Viagra has been used to treat male erectile dysfunction with generally minor side effects. It is now being increasingly used for other indications including pulmonary hypertension.

Beyond Viagra, there are a number of other PDE inhibitors that are used clinically. In fact, the classic drugs papaverine and dipyridamole were used clinically before their effects on PDEs were known. Caffeine and theophylline (a compound found in tea) are also PDE inhibitors. However, all of these drugs most likely have multiple targets, making conclusions regarding the roles of PDEs in processes that are sensitive to these agents difficult to interpret. Certainly, some of their effects are due to their actions on adenosine receptors.

The PDE3 inhibitor, cilostazol, has been used as an antithrombotic agent and is currently being used in patients being treated for intermittent claudication. Cilostazol is also used for the prevention of [restenosis](#) after treatments such as angioplasty. Another PDE3 selective inhibitor, milrinone, has been used in the treatment of congestive heart failure. Milrinone also has been shown to increase the conductance of the CFTR transporter in vitro.

PDE4 inhibitors may also serve as antiinflammatory agents. Older PDE4 inhibitors tended to be limited by their emetic side effects, but newer drugs are now in clinical trials and may have milder side effects. These drugs (Ariflo; GlaxoSmithKline, and Roflumilast; Byk Gulden) are being tested for the treatment of asthma and chronic obstructive pulmonary disease with some success.

Dipyridamole is a PDE5/PDE6 selective inhibitor that is used widely in conjunction with aspirin to reduce clotting and prevent stroke. More recent studies with a fixed combination of these two drugs (Aggrenox) has been shown in the recent European Stroke Prevention Study 2 to be of greatly added benefit over aspirin alone for prevention of recurrent stroke.

Finally, IBMX is a methylxanthine derivative that has long been used in vitro as a general PDE inhibitor. IBMX is effective at inhibiting most PDEs, with IC₅₀s of 2–50 μ M. However, the more recently cloned PDEs, PDE8, and PDE9, are IBMX-resistant. It is therefore important to keep this in mind when using IBMX to investigate potential roles for PDEs. A rather comprehensive review of the PDE field has appeared recently[1].

- Adenylyl Cyclases
- Guanylyl Cyclase
- Smooth Muscle Tone Regulation

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Phosphoinositol Kinases

- Phospholipid Kinases

Phospholipases

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Definition

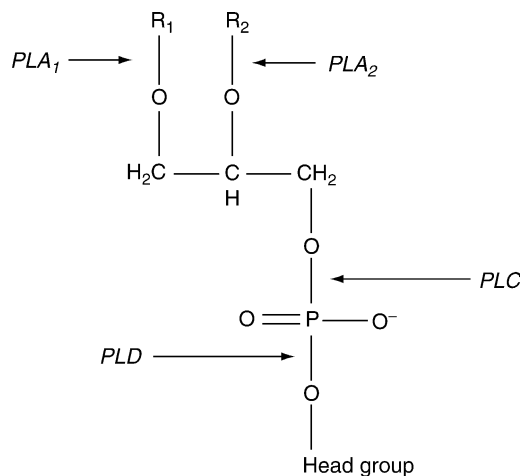
Enzymes that hydrolyze ► phospholipids

Basic Characteristics

Phospholipases are widely distributed in nature and carry out many important cellular functions. They are classified into four types: A₁, A₂, C and D. Phospholipase A₁ and A₂ act on the ester bonds that link fatty acids to the sn-1 and sn-2 positions of the glycerol backbone of phospholipids (Fig. 1). Their action generates free fatty acids and lysophospholipids. Phospholipase C acts on the phosphodiester bond that links the headgroup of the phospholipid to the glycerol backbone. It yields phosphorylated headgroups and ► diacylglycerol (DAG). Phospholipase D acts on the other side of the phosphodiester bond to yield free headgroups and ► phosphatidic acid (PA). Phospholipases alter cell activities through their effects on membrane phospholipids. Their products also influence cellular functions by acting as intracellular and extracellular messengers. Thus the lipids (fatty acids, DAG, PA) released as a result of phospholipase activity can alter the activity of enzymes and other cellular proteins or they can be metabolized to other lipids, some of which are released and exert diverse effects (eicosanoids, lysophosphatidic acid). Mammalian phospholipase C acts on inositol phospholipids, principally ► phosphatidylinositol 4,5-bisphosphate (PIP₂) and the phosphorylated headgroup primarily released by phospholipase C action (inositol 1,4,5-trisphosphate, IP₃) plays a major role in Ca²⁺ mobilization in the cell, with subsequent important physiological consequences. As expected from their important physiological functions, many phospholipases are highly regulated by hormones, neurotransmitters, growth factors and cytokines.

Phospholipase A1

Phospholipase A1 (PLA₁) is widely distributed. It has not been studied extensively and is not known to be



Phospholipases. Figure 1 Generic phospholipid with sites of action of phospholipases shown.

regulated. Its major substrates are PA, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and it releases mainly saturated or monounsaturated fatty acids, which are predominantly present at position sn-1 of these phospholipids. Through the combined action of PLA₁ and lysophospholipase, arachidonic or other unsaturated fatty acids can be released from the sn-2 position also. A PA-preferring PLA₁ isozyme has been cloned. It has a molecular mass of 98 kDa and is expressed in brain and testis. No other isozymes have been cloned. The function of PLA₁ relates to phospholipid remodeling, i.e. the substitution of one fatty acid by another, which it accomplishes in combination with lysophospholipid acyltransferase.

Phospholipase A₂

Phospholipase A₂ (PLA₂) occurs in several forms as the products of different genes (Tables 1 and 2). Some forms are of low molecular mass (<14 kDa) and are secreted by cells, whereas others are of higher mass (26–114 kDa) and are mainly located in the cytosol [1]. The smaller forms are components of snake and bee venoms, but some are also present in mammalian tissues and are secreted into pancreatic juice and synovial fluid [1]. They have a rigid crystal structure due to their high disulfide bond content (Table 1). Catalysis involves a conserved His that, together with a conserved Asp, polarizes a bound H₂O to attack the substrate carbonyl group. They require millimolar Ca²⁺ for activity. The Ca²⁺ is bound to a conserved glycine-rich loop and interacts with the conserved Asp to stabilize the transition state. Secretory PLA₂s (sPLA₂s) act predominantly on PC and PE and have been classified into several groups (I, II, III, V and X) based on sequence differences (Table 1). For conciseness, only the mammalian groups will be described. Group I sPLA₂ is present in pancreatic acinar cells as a proenzyme form. It is released into pancreatic juice and is converted by trypsin to the active form, which is involved in digestion. Group II sPLA₂ exists in several subgroups and is present in synovial fluid and many tissues. Group III sPLA₂ is present in several human tissues and has a preference for phosphatidylglycerol. Groups V and X are also widely distributed and are active on PC and PE. Serum levels of sPLA₂s are elevated in inflammatory conditions and trauma, and secretion of these isozymes into other body fluids occurs in specific diseases, e.g., pancreatitis, inflammatory bowel disease and arthritis. The secretion and expression of sPLA₂s is increased by pro-inflammatory cytokines, e.g., tumor necrosis factor α and certain interleukins, resulting in the production of arachidonic acid that is subsequently converted to prostaglandins and other eicosanoids.

The cytosolic forms show no sequence homology to the secreted forms and have a different catalytic mechanism [1]. They are widely distributed and occur

Phospholipases. Table 1 Classification of low molecular mass phospholipase A₂ isozymes that use catalytic His

Group	Sources	Molecular Mass (kDa)	Disulfides (no.)
I A, B	Snake venom, pancreas	13–15	7
II A–F	Snake venom, synovial fluid, pancreas, testis, spleen, brain, heart, uterus	13–17	6–8
III	Bee, lizard, scorpion, human	15–18	5
V	Heart, lung, macrophages	14	6
IX	Snail venom	14	6
X	Spleen, thymus, leukocytes	14	8
XI A, B	Rice	12–13	6

Adapted from Six and Dennis [1]

Phospholipases. Table 2 Classification of higher molecular mass phospholipase A₂ isozymes that use catalytic Ser

Group	Sources	Molecular Mass (kDa)	Ca ²⁺ Requirement
IV A–C	Platelets, lymphocytes, kidney, pancreas, liver, heart, brain, skeletal muscle	61–114	<μM ^a
VI A, B	Macrophages, lymphocytes, heart, skeletal muscle	84–90	0
VII A, B	Plasma, kidney, liver	40–45	0
VIII A, B	Brain	26	0

Adapted from Six and Dennis [1]

^aGroup IVC does not require Ca²⁺

as either Ca²⁺-dependent and Ca²⁺-independent forms (Table 2). Ca²⁺-dependent PLA₂ (Group IV or cPLA₂) occurs in three isoforms (α , β , γ). cPLA₂ α is regulated by cytokines and growth factors and plays a major role in the regulated production of arachidonic acid. It shows marked specificity for phospholipids containing arachidonic acid in position sn-2 and also exhibits

lysophospholipase activity. The arachidonic acid produced is subsequently metabolized to a variety of eicosanoids (e.g., prostaglandins, prostacyclin, thromboxanes, leukotrienes) that contributes to inflammation and a large variety of other physiological responses. These include changes in blood flow, platelet function, smooth muscle contractility, renal function, endocrine responses and gastrointestinal secretion. These effects may be positive or negative, depending on the specific eicosanoid and target tissue.

cPLA₂α (85 kDa) has been much studied and contains a Ca²⁺-lipid-dependent binding (CaLB) domain in its N-terminus [1]. The structure of this domain is similar to that of the C-2 Ca²⁺-binding domains found in ►protein kinase C, phospholipase Cδ and synaptotagmin. The catalytic mechanism is different from that of the low molecular mass PLA₂s and involves a Gly-Leu-Ser-Gly-Ser sequence, which resembles that seen in many serine esterases and neutral lipases. The central Ser (Ser²²⁸) serves as the active site nucleophile and catalyzes hydrolysis in association with Asp⁵⁴⁹. Like other serine esterases in which the nucleophilic serine is on a “nucleophilic elbow” (a turn between a β strand and α-helix), cPLA₂ has a central β-sheet with Ser²²⁸ located within this “elbow.” However, other structural features of the catalytic site are quite different from those of other serine esterases. Surprisingly, the catalytic site is buried quite deeply in the catalytic domain core suggesting that the enzyme must become embedded in the lipid bilayer.

An increase in Ca²⁺ in the micromolar range induces translocation of cPLA₂ to the nuclear membrane. The translocation involves the CaLB domain that presents the catalytic domain to the phospholipid substrate, resulting in increased arachidonic acid release. The enzyme is also phosphorylated and activated by mitogen-activated protein kinases (MAPKs). The phosphorylation is catalyzed by both extracellular signal-regulated kinase (ERK) and p38 MAPK, and involves a specific residue (Ser⁵⁰⁵). This Ser residue is located within the catalytic domain in close proximity to the C-2 Ca²⁺ binding domain. It has been proposed that phosphorylation of Ser⁵⁰⁵ improves the positioning of the catalytic site in relation to the lipid bilayer. In addition to its rapid activation by agonists that elevate cytosolic Ca²⁺ and activate MAP kinase, cPLA₂ can be regulated at the transcriptional level by the proinflammatory cytokines interleukin 1 and tumor necrosis factor α, whose action is inhibited by glucocorticoids. Other factors (macrophage colony-stimulating factor, lipopolysaccharide, epidermal growth factor) also activate transcription of cPLA₂.

The Ca²⁺-independent cytosolic PLA₂ (iPLA₂ or Group VI) isozymes are widely distributed and located predominantly in the cytosol [1], although there are some membrane-associated forms (Table 2). Their molecular masses range between 84 and 90 kDa due

to multiple splice variants, and some forms contain ankyrin repeats. They contain the lipase consensus sequence and the active Ser found in cPLA₂s. They are involved in phospholipid fatty acid remodeling and may be involved in arachidonic acid release and prostaglandin formation, although this is limited compared with other PLA₂ isozymes.

Two other groups of PLA₂s (Groups VII and VIII) inactivate platelet-activating factor (PAF) [1]. They are also called PAF-acetylhydrolases and are highly specific for PAF or PAF analogues with short-chain oxidized fatty acids in position sn-2. PAF is a modified form of PC in which the sn-1 chain is linked by an ether linkage, and an acetyl group is present at sn-2. It has many potent effects including vasodilation, platelet activation, chemotactic action and smooth muscle contraction. These types of PLA₂ have the characteristic lipase catalytic sequence of cytosolic PLA₂s, but are of lower molecular mass (26–45 kDa). They exist as either intracellular or secreted forms, and are found in many tissues.

Phospholipase C

In contrast to phospholipases of the A type, which have broad substrate specificity, phospholipase C (PLC) in mammalian cells acts only on inositol-containing phospholipids, in particular PIP₂, which is cleaved to form IP₃ and DAG (►PI response). Mammalian PLC occurs as four different isozymes (β, γ, δ and ε) and there are several subtypes of these (β1–β4, γ1, γ2, δ1–δ4) [2]. The β1, β3, γ1, δ1, δ4 and ε isozymes are widely distributed, whereas the β2 and γ2 forms are found predominantly in hematopoietic cells, and the β4 isozyme is confined to retina and brain. The β and γ isozymes are highly regulated by different agonists, whereas the δ isozymes are not subject to agonist control, but are stimulated by a rise in cytosolic Ca²⁺ [2]. The β isozymes are activated by α- or βγ-subunits released from heterotrimeric proteins of the G_q and G_{i/o} families as the result of activation of certain receptors. The γ isozymes are activated by growth factor receptors that encode tyrosine kinase activity and by cytokines that induce tyrosine phosphorylation of their receptors. The PLCε isozyme contains a Ras guanine nucleotide exchange factor domain (GRF CDC25) and two Ras binding domains. It is directly activated by active Ras, which also causes its membrane translocation.

All PLC isozymes have conserved catalytic domains designated X and Y, and a C2 domain similar to that in cPLA₂ (Fig. 2). In addition, the β, γ and δ isozymes have pleckstrin homology (PH) domains and EF-hand domains located in the N-terminal region. The γ isozymes differ in that they have Src homology domains (SH2 and SH3) and an additional PH domain split by the SH domains. The β and γ isozymes are of 140–155 kDa mass, whereas the δ isozymes are smaller (85 kDa) and the σ isozyme is larger (240 kDa).

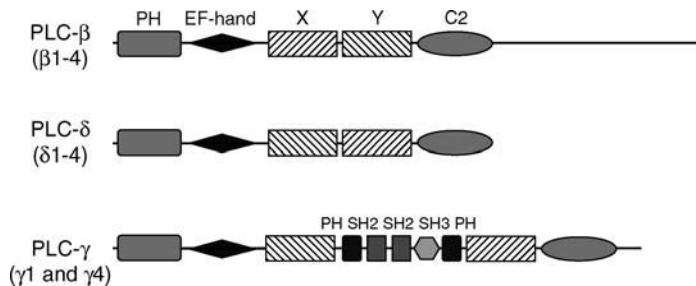
The three-dimensional structure of PLC- δ 1 has been defined. The catalytic domain is in the form of an α/β or TIM barrel (discovered first in triosephosphate isomerase). The active site is in the cleft of the barrel and contains a coordinated Ca^{2+} that is required for catalysis. The proposed general mechanism is general base/general acid catalysis. Membrane binding of the enzyme depends on the PH domain, which is proposed to tether the enzyme through PIP_2 binding. The C2 domain is proposed to fix the catalytic domain so that it can penetrate the membrane, allowing PIP_2 hydrolysis to proceed.

The β isozymes are activated by G protein-coupled receptors through two different mechanisms [2]. The first involves activated α -subunits of the G_q family of heterotrimeric G proteins (G_q , G_{11} , G_{14} , $G_{15/16}$). These subunits activate the β 1, β 3 and β 4 PLC isozymes through direct interaction with a sequence in the C terminus. The domain on the $G_q\alpha$ -subunit that interacts with the β isozymes is located on a surface α -helix that is adjacent to the Switch III region, which undergoes a marked conformational change during activation. The second mechanism of G protein activation of PLC β isozymes involves $\beta\gamma$ -subunits released from $G_{i/o}$ G proteins by their pertussis toxin-sensitive activation by certain receptors. The $\beta\gamma$ -subunits activate the β 2 and β 3 PLC isozymes by interacting with a sequence between the conserved X and Y domains.

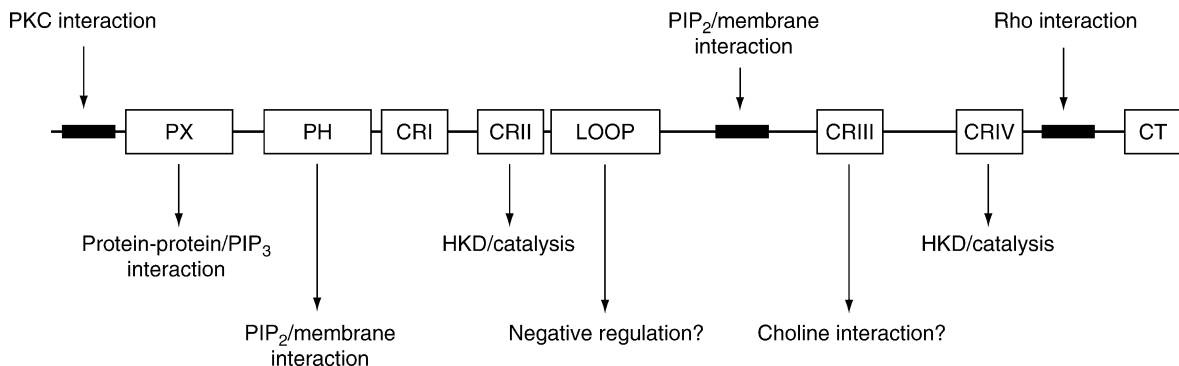
The physiological importance of PLC activation is to cleave PIP_2 into IP_3 and DAG. IP_3 induces the release of Ca^{2+} from Ca^{2+} stores in the endoplasmic reticulum, which results secondarily in increased Ca^{2+} influx into the cell. The resultant increase in cytosolic Ca^{2+} causes a variety of physiological responses including smooth muscle contraction in many organs, secretion of cellular constituents in many cells and glycogen breakdown and other metabolic responses in liver and other organs. The DAG that accumulates in the plasma membrane as a result of PLC activation causes membrane translocation and activation of most isozymes of protein kinase C (PKC). This kinase acts on many membrane proteins (e.g., receptors, ion channels, transporters) to modulate their activities and modify certain physiological responses.

Phospholipase D

Phospholipase D is widely distributed in bacteria, fungi, plants and animals, and is present in almost all mammalian cells [3]. In mammals, it occurs as alternatively spliced products of two genes (PLD1 and PLD2) (Fig. 3). Most mammalian cells express different levels of both isoforms. Both PLD1 and PLD2 have four conserved sequences (I-IV), and sequences I and IV contain the HXXK₄D (HKD) motif that is characteristic of the PLD superfamily, which includes bacterial endonucleases, phospholipid synthases, viral envelope



Phospholipases. Figure 2 Representation of the domains in the major isozymes of phospholipase C (adapted from [2]).



Phospholipases. Figure 3 Representation of the domain structure of phospholipase D1 (adapted from [4]).

proteins and a murine toxin (Fig. 3) [3]. Both HKD domains are required for catalytic activity and they dimerize to form the catalytic center. Catalysis occurs in a two-step reaction involving the two His residues of the dimer [3]. The first step is a nucleophilic attack by one His on the substrate phosphorus to produce a phosphatidyl-enzyme intermediate. The second step involves the other His that protonates the oxygen of the leaving group. Both PLD isoforms require PIP₂ for activity, but are distributed in different cellular sites and are differentially regulated. PLD1 is activated in vitro by the α and β isozymes of PKC and by members of the Rho and ARF families of low Mr G proteins [3]. Combinations of these proteins produce synergistic activation of the enzyme. In contrast, PLD2 is not regulated by these factors in vitro. The interaction site for PKC α is at the N-terminus of PLD1, while that for RhoA is at the C-terminus. The binding site for ARF is unknown.

PLD is highly regulated in vivo by hormones, neurotransmitters and other G protein-coupled agonists, and also by growth factors and cytokines. The regulation is not direct but involves signaling through PKC and Rho family proteins. The enzyme can be phosphorylated on Tyr and Ser/Thr residues in vivo, but the role of these phosphorylations in its regulation is unclear. The cellular roles of PLD include the regulation of Golgi function, exocytosis, endocytosis, the actin cytoskeleton, superoxide production and growth. It has been proposed that the role of ARF in vesicle trafficking in the Golgi involves not only coatamer formation, but also PLD activation, which stimulates vesicle budding through PA formation. PLD is implicated in catecholamine secretion, endocytosis of membrane receptors and glucose transport. The rearrangement of the actin cytoskeleton resulting in stress fiber formation also depends on PLD activity. The activation of NADPH oxidase that yields superoxide in neutrophils involves PA formation via PLD activity, and PLD has been implicated in ERK activation in some cells.

Drugs

Phospholipase A₂

Glucocorticoids inhibit cPLA₂ at the level of transcription and this is part of their anti-inflammatory action. Antimalarial drugs (mepacrine, aminoglycosides and polyamines) inhibit PLA₂ activity, but they are too non-specific to be therapeutically useful. This is also true of covalent-modifying PLA₂ agents such as manoalide and *p*-bromophenacyl bromide, which are selective for sPLA₂ in vitro. Another agent 3-(3-acetamide-1-benzyl-2-ethylindol-5-oxy) propane sulfonic acid (LY311727) inhibits sPLA₂ selectively in cell studies, but there have been no reports of its use in vivo. Arachidonyl trifluoromethyl ketone and methylarachidonyl fluorophosphonate are

potent cPLA₂ inhibitors. However, they are too toxic and insufficiently specific to be useful therapeutically. Bromoenol lactone, which is a potent inhibitor of cPLA₂, has actions on other lipid-metabolizing enzymes and therefore limits its use in vivo.

Phospholipase C

There are no specific inhibitors of PLC, but compounds that interact with PIP₂, e.g., neomycin can reduce its activity. However, such drugs interfere with other signaling processes involving this lipid. The aminosteroid 1-[6-[[17 β -3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (U-73122) has been reported to inhibit PLC, but has other effects related to cell Ca²⁺ homeostasis.

Phospholipase D

There are no specific inhibitors of PLD. Xanthogenate tricyclodecan-9-yl (D609) and 1-*O*-octadecyl-2-*O*-methyl-sn-glycero-3-phosphocholine (ET-18-OCH₃) lack specificity since they act by competing with substrate (phosphatidylcholine). Primary alcohols such as ethanol, propanol and butanol inhibit the actions of PLD by reducing the formation of PA through the transphosphatidyl reaction that generates phosphatidylalcohols. Since PA is the primary signaling molecule produced by PLD action, the cellular actions of this lipid are curtailed.

► Phospholipid Kinases

► Transmembrane Signaling

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Phospholipids

Phospholipids are a major component of all biological membranes together with glycolipids and cholesterol. Due to their polar nature, i.e. hydrophilic head and hydrophobic tail, phospholipids form in water vesicles or liposomes.

Phospholipid Kinases

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Synonyms

Phosphoinositol kinases; Phosphatidylinositol kinases;
Phosphatidylinositide kinases

Definition

► **Phospholipid** kinases comprise a family of enzymes that phosphorylate ► **phosphatidylinositol** and phosphatidylinositides at positions 3', 4' or 5' but not at positions 2' and 6' of the inositol ring (Fig. 1). Phosphatidylinositides represent approximately 1% of all membrane lipids such as those of the plasma, nuclear and endomembranes. In unstimulated cells, more than 90% of all phosphoinositides correspond to unphosphorylated phosphatidylinositol (PtdIns), whereas the remaining 10% consist of roughly equal amounts of phosphatidylinositol 4-phosphate (PtdIns(4)P) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) [1]. PtdIns and its derivatives are referred as phosphoinositides or PIs. Initially, it was assumed that these phosphoinositides function exclusively as precursors for signalling molecules. For instance, PtdIns(4,5)P₂ serves as a substrate for phospholipase C and phosphoinositide 3-kinases (PI3K). Interestingly, PtdIns(4,5)P₂ also interacts with intracellular proteins in a regulatory manner, thereby affecting their localization and activity. In resting cells, less than 0.25% of the phosphoinositides are 3'-phosphorylated. Stimulation of cells with ligands that activate receptor tyrosine kinases or ► **G protein-coupled receptors** (GPCRs) results in the rapid and transient phosphorylation of PtdIns(4,5)P₂ to phosphatidylinositol 3,4,5-triphosphate PtdIns(3,4,5)P₃. This 3'-phosphorylated phosphoinositide behaves as a typical second messenger. Hence, the responsible phosphoinositide 3-kinases are considered as important regulatory molecules of the cell.

All phosphoinositides are found in the cytosolic half of the lipid bilayer of the plasma or intracellular compartment membranes (left part). The different kinases acting on phosphoinositides in mammalian cells are shown in solid lines and the phosphoinositide 3-kinases, in bold. The phosphoinositides counterpart pathways catalysed by known phosphatases are represented by dashed lines. The best known phosphatases are PTEN (Phosphatase and tensin homolog deleted on chromosome 10) and SHIP (SH₂ domain-containing inositol 5-phosphatase).

Basic Characteristics

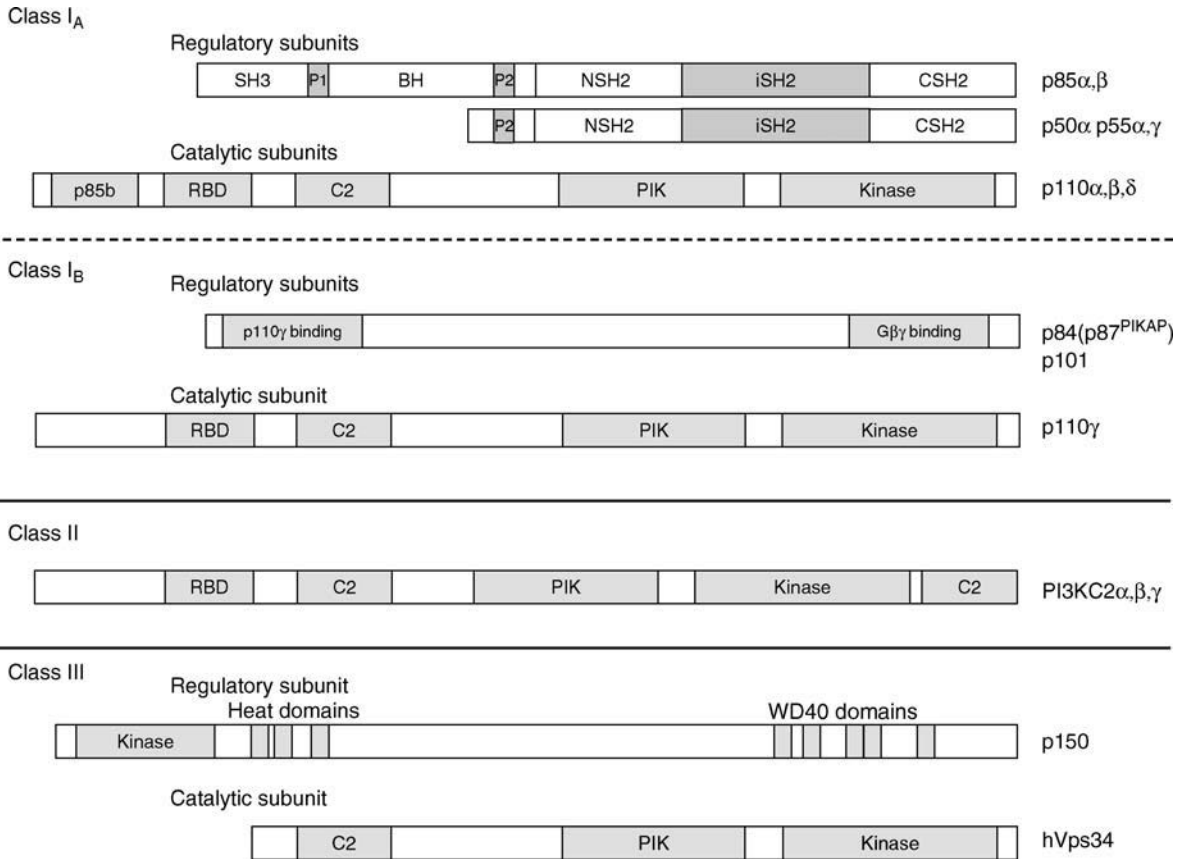
Cellular phosphoinositide concentrations are under tight control by phospholipid kinases and phosphatases. Phospholipid kinases preferentially phosphorylate distinct positions of the inositol ring and hence are subdivided into phosphoinositide 3-kinases (PI3Ks), phosphoinositide 4-kinases (PI4Ks), and phosphoinositide 5-kinases (PI5Ks) that phosphorylate PIs on position 3', 4' and 5', respectively. In a canonical pathway, PtdIns(4,5)P₂ is generated from PtdIns(4)P by the enzymatic activity of phosphatidylinositol 4-phosphate 5-kinase (PIP5K) (Fig. 1). Additional pathways are likely to be discovered.

Among the described phosphoinositides, PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂ have been recognized as important intracellular mediators of signalling processes. These molecules act as second messengers generated after stimulation of cells by extracellular stimuli. They are involved in cell survival pathways, the regulation of gene expression, cell metabolism and cytoskeletal rearrangements involved in cell motility [2]. Correspondingly, the enzymes producing PtdIns(3,4,5)P₃ have been implicated in major human diseases such as diabetes, cancer and inflammatory processes. This has generated high interest in establishing specific pharmacological tools to interfere with PtdIns 3-dependent pathways. Based on their substrate specificity, phosphoinositide 3-kinases (PI3K) are subgrouped into three classes (Fig. 2).

Shown are schematic representations of the catalytic and regulatory subunits of PI3Ks. SH2, SH3 (src-homology domain 2 and 3), P1, P2 (proline rich region 1 and 2), RBD (ras binding domain) represent protein-protein interaction domains. The C2 domain is a membrane-binding domain that was first identified as a calcium-binding domain in protein kinase C (PKC). The PIK (phosphoinositide kinase) domain is shared by all the lipid kinases; p85b indicates the binding site for the regulatory subunits.

Classification of PI3Ks

All class I PI3Ks are heterodimeric enzymes composed of a 110 kDa catalytic subunit (with the isoforms p110 α , β , δ or γ) that associates with a regulatory subunit. Although the class I PI3Ks are capable of phosphorylating PtdIns, PtdIns(4)P and PtdIns(4,5)P₂ in vitro, it appears that they only use PtdIns(4,5)P₂ as a substrate in vivo. Receptor-induced formation of PtdIns(3,4,5)P₃ from PtdIns(4,5)P₂ seems restricted to the enzymatic activity of class I kinases. Class I_A PI3K catalytic subunits are relatively unstable and form a complex with either one of five different regulatory subunits (p85 α , p55 α , p50 α , p85 β or p55 γ), which contain ► **two src-homology 2 (SH2-domains)**. These SH2-domains specifically recognize phosphorylated tyrosine residues that are present on activated growth



Phospholipid Kinases. Figure 2 Classification of phosphoinositide 3-kinases.

factor receptors or on growth factor receptor substrates. This interaction results in a translocation of the cytosolic heterodimeric enzyme to the inner leaflet of the plasma membrane, thus bringing the catalytic subunit p110 close to its lipid substrate PtdIns(4,5)P₂. In addition, the interaction with the tyrosine-phosphorylated receptor induces a conformational change of p85, which disinhibits the enzymatic activity of the p110 catalytic subunit. The p85 subunits contain characteristic protein domains which allow multiple protein–protein interactions (Fig. 2). For instance, the inter-SH2-region (iSH2) of p85 mediates the interaction with the catalytic p110 subunit. Interestingly, p85 may possess also a PI3K-activity independent role. Indeed, this regulatory subunit has been implicated, independently of the catalytic subunit p110, in cell migration and cytokinesis. All class I PI3Ks are also assumed to be under control of monomeric [▶Blue proteins](#). There is evidence that the different Ras isoforms activate PI3Ks with different potencies. The impact of this type of regulation is currently not well understood. Very little is known in molecular terms about the apparent preference of a given receptor for a specific catalytic subunit. One may speculate that both the growth factor receptor interacting with p85 and the Ras protein interacting with p110 can

influence the assignment of a specific p110 isoform to a specific signalling pathway. Class I_A PI3Ks are mainly involved in the regulation of cell growth and proliferation [2]. Furthermore, they are essential for mediating insulin functions, and have been linked to diabetes mellitus type 2. An increase of the class I_A PI3K activity has been found in a number of different human cancers.

Class I_B PI3Ks signal downstream of GPCRs. The p110 γ catalytic subunit is associated with one of two regulatory subunits, p101 or p84 (also known as p87^{PIKAP}). p101 and p84 are structurally distinct from class I_A PI3K adaptor proteins. Similar to class I_A PI3Ks, unstimulated class I_B PI3K γ is predominantly localized in the cytosol. Upon GPCR activation, G $\beta\gamma$ dimers directly bind PI3K γ and induce a recruitment to the plasma membrane and a subsequent activation of PI3K γ . p84-containing PI3K γ is less sensitive towards G $\beta\gamma$ than p101-containing PI3K γ . The physiological significance of this difference is not known. In addition to its predominant expression in the haematopoietic cells, PI3K γ is also found in other organs and tissues including cardiac and vascular tissues.

Two other classes of PI3Ks have been less intensively investigated. Class II PI3Ks are subdivided into PI3K-C2 α , PI3K-C2 β and PI3K-C2 γ . They are large

monomeric enzymes of 170–210 kDa molecular weight that phosphorylate PtdIns to PtdIns(3)P. In contrast to class I PI3K mainly cytosolic, class II PI3Ks are predominantly found in membrane fraction of cells including plasma and intracellular membranes [1]. They respond to a wide array of stimuli, such as chemokines, insulin and growth factors through mechanisms that are not yet well defined. Based on their potential role in cell survival and cell migration, class II PI3Ks are also considered as potential targets for anticancer therapies.

The vacuolar protein-sorting protein (Vps34p) represents the prototypical class III PI3K. Vps34p was initially identified in *Saccharomyces cerevisiae*. In mammalian cells, hVps34 is involved in the regulation of ►autophagy, a conserved lysosomal degradation pathway. A serine/threonine kinase, i.e. p150, is known to form a heterodimer with hVps34, and to be responsible for the membrane association of this PI3K. A Bcl-2-interacting protein named Beclin 1 forms a complex with hVps34/Class III PI3K to generate PtdIns(3)P, which is thought to be important in mediating the localization of other proteins to preautophagosomal membranes. Interestingly, Beclin is found monoallelically deleted in a high number of humans cancers. Currently, investigations are underway to define the role of autophagy in human diseases like cancer, pathogen infection and neurodegeneration. Because of its role in autophagy, type III PI3K may be a potential therapeutic target for the treatment of these diseases [3].

Protein Kinase Activity of PI3K

In all the known catalytic subunits of PI3K, the C-terminal kinase domains show considerable similarity with classical serine/threonine kinases. Indeed, PI3Ks can function as dual-specific enzymes and exhibit a protein kinase activity in addition to their lipid kinase activity. Novel aspects of PI3K regulation were revealed by the discovery that the PI3K lipid kinase activity can be modulated by autophosphorylation of the catalytic subunit p110 and/or by trans-phosphorylation of the associated regulatory p85. Accordingly, it became apparent that both enzymatic qualities, i.e. the lipid as well as the protein kinase activities, are required for certain physiological processes. For instance, the endocytosis of β -adrenergic receptors requires both lipid and protein kinase activities of PI3K γ . To date, however, very few protein substrates for PI3K are known. Recently, a surprising role for PI3K γ as a scaffolding protein has emerged from cardiovascular studies of genetically engineered mice expressing a catalytically inactive point mutant of PI3K γ . These results have led to the proposal that PI3K γ regulates cardiac contractility independent of its kinase activity.

Downstream Effectors of PI3Ks

The importance of PI3Ks arises from the fact that numerous important signalling molecules need to be

localized to the plasma membrane to fulfill their functions. Binding to 3'-phosphorylated phosphoinositides constitutes a major mechanism of membrane translocation for the signalling molecules. The prototypical phosphoinositide binding domain is the ►pleckstrin homology-domain (PH-domain), which binds to the second messengers PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂ with different affinities (Fig. 3). A PH-domain protein known to play a pivotal role in PI3K signalling is protein kinase B (PKB), also called *Akt* [4]. Plasma membrane recruited and activated PKB triggers multiple signalling cascades implicated in an array of normal and pathophysiological cell processes, such as inflammation, metabolism and tumorigenesis (Fig. 3).

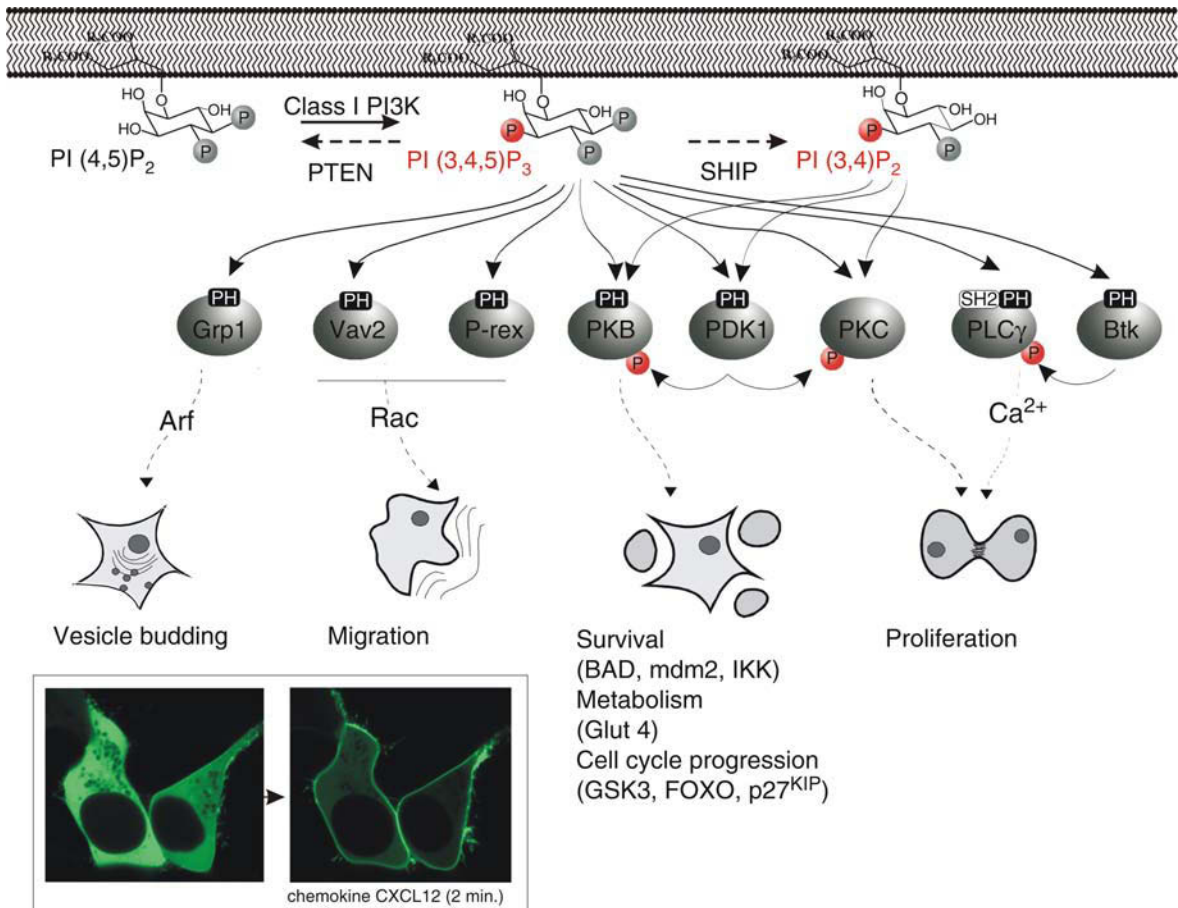
Bottom left panel: Visualization of the translocation of GFP-fused Grp1 PH-domain in living cells. Human embryonic kidney cells (HEK293) were stimulated with the chemokine CXCL12. The GFP-fused Grp1 PH-domain protein translocates from the cytosol to the plasma membrane, reflecting an increased PI3K γ -mediated PtdIns(3,4,5)P₃ production. HEK293 were transfected with the chemotactic GPCR (CXCR4), PI3K γ and GFP-fused Grp1 PH-domain.

Numerous other PH-domain containing proteins are implicated in important physiological processes, such as the guanosine nucleotide exchange factors (GEFs) Vav and P-Rex. GEFs exchange GDP for GTP and lead to an activation of small GTPases. Vav and P-Rex regulate the activity of the small GTPase Rho/Rac and are involved in cytoskeletal remodelling and cell motility. Grp1, a GEF for the small GTPase Arf, is necessary for vesicle budding (Fig. 3).

PtdIns(3)P generated by class II or III PI3Ks interacts with the FYVE Zinc finger domains (acronym of FYVE: *Fab1p*, *YOTB*, *Vac1p* and *EEA1*) as well as PX domains (a domain originally identified as a common motif in the p40phox and p47phox of the neutrophil NADPH oxidase complex). Other phosphoinositides-binding domains have been identified. Among them, are FERM domains (band four-point one, ezrin, radixin, moesin) that link cytoskeleton to the plasma membrane, or ENTH domains (epsin N-terminal homology) that play a role in endocytosis.

Drugs

As described above, PI3Ks are critical for many signalling pathways that control growth and survival of the cells. Not surprisingly, PI3Ks are regarded as attractive drug target for cancer therapy and for the treatment of other human diseases. The development of PI3K deficient mice has contributed to our understanding of specific physiological roles of the various catalytic and regulatory subunits in mammals. The deletion of either p110 α or p110 β genes in mice results in embryonic lethality, thus limiting the analyses of the post-embryonic functions of these catalytic PI3K isoforms. In contrast, mice lacking the genes encoding for PI3K δ and γ are



Phospholipid Kinases. Figure 3 Role of PH-domain containing effectors of class I PI3Ks in various cellular processes.

viable. The studies of characteristics of PI3K γ - and δ -deficient mice have revealed the importance of these two isoforms in certain immune cell functions, such as the development of T and B cells, leukocyte migration, mast cell degranulation and oxidative burst of neutrophils. The classical PI3K inhibitors ►Wortmannin and LY294002 show very little selectivity for the different PI3K isoforms. A recent challenge for pharmaceutical and academic groups alike consists in identification of isoform-selective small-molecule PI3K inhibitors. Such inhibitors could be used for treatment of cancer, neurodegeneration or inflammatory diseases. Recently, very promising studies show that new inhibitors targeting specifically PI3K γ reduce the severity of inflammatory diseases in mice model of rheumatoid arthritis and lupus [5]. These isoform-selective inhibitors could potentially be of great use for the treatment of human inflammatory diseases.

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Phospholipid Phosphatases

Phospholipid phosphatases are enzymes such as SHIP (SH2-domain containing inositolide-5-phosphatase) or PTEN (phosphatase and tensin homolog deleted on chromosome 10) which dephosphorylate phosphoinositides. Whereas SHIP removes phosphate from the 5'

position, PTEN dephosphorylates the 3' position of the inositol. Therefore, PTEN has opposite effects to phosphatidylinositol (PI) 3-kinases and is considered to be a tumor suppressor.

- Phosphatases
- Phospholipid Kinases

Phosphorylation

Phosphorylation is the reversible process of introducing a phosphate group onto a protein. Phosphorylation occurs on the hydroxyamino acids serine and threonine or on tyrosine residues targeted by Ser/Thr kinases and tyrosine kinases respectively. Dephosphorylation is catalyzed by phosphatases. Phosphorylation is a key mechanism for rapid posttranslational modulation of protein function. It is widely exploited in cellular processes to control various aspects of cell signaling, cell proliferation, cell differentiation, cell survival, cell metabolism, cell motility, and gene transcription.

Phosphotyrosine-binding Domains

Synonyms

PTB

Definition

Noncatalytic phosphotyrosine binding (PTB) domains are 100–150 residue modules, which bind Asn-Pro-X-Tyr motifs. PTB-domain binding specificity is determined by residues at the amino-terminal side of the phosphotyrosine. In most cases, the tyrosine residue must be phosphorylated in order to mediate binding. PTB domain containing proteins are often found in signal transduction pathways.

- Growth Factors

Photoaging

Photoaging and photodamage (dermatoheliosis) are terms used interchangeably to describe chronic changes in the appearance and function of the skin caused by

repeated sun exposure rather than by the passage of time (the latter is called intrinsic or chronologic aging). Epidemiologic and laboratory evidence indicates that sun exposure and other sources of UV radiation (UVR) play the major role in causing the undesirable skin changes of fine and coarse wrinkles, roughness, laxity, mottled pigmentation, actinic lentigines, actinic keratoses, leathery texture/coarseness, scaling/xerosis, sallowness, and telangiectasia. Cigarette smoking is the only other environmental factor that has been related to the development of changes in the skin associated with aging.

Photoaging and photodamage are serious medical problems, not just cosmetic or aesthetic concerns, because prevention of photoaging and photodamage may prevent the progression of changes toward skin cancer.

The use of sunscreens that protect against UVB and UVA should be encouraged. There is no safe way to tan!

- Retinoids

Phthalate Esters

Di- and mono-esters of phthalic acid, an orthodicarboxylic acid derivative of benzene. These compounds are widely used as industrial plasticizers to coat polyvinylchloride surfaces of plastics used in food packaging and medical devices (iv drip bags, blood storage bags, etc.) and are common environmental contaminants. Several phthalate mono-esters are peroxisome proliferator chemicals and can activate the peroxisome proliferator-activated receptor PPAR.

- Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes
- Peroxisome Proliferator-Activated Receptor PPAR

Phylloquinones

- Vitamin K

Physical Dependence

Physical dependence on a substance is characterized by the desire or compulsion to continue taking the

substance, a tendency to increase the dose due to increasing tolerance, a dependence on the properties of the substance as manifested by somatic withdrawal symptoms and a negative influence on the individual and society.

►Drug Addiction/Dependence

PI Response

Hormonal factors and other stimuli by activating phospholipase C- β or - γ isoforms stimulate the breakdown of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate and diacylglycerol, a reaction called PI response.

- Phospholipases
- Transmembrane Signalling

PIAS

Beside other functions in signal transduction, protein inhibitors of activated STAT suppress the DNA-binding activity of STAT proteins.

►PIAS Proteins

PIAS Proteins

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Definition

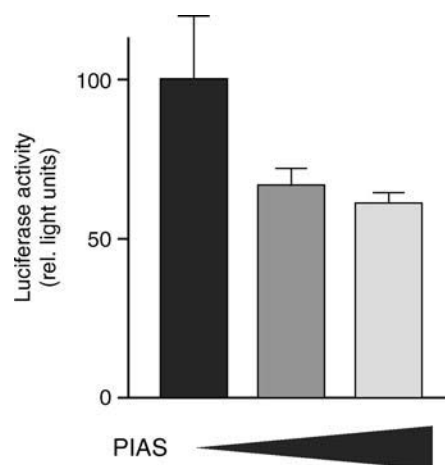
►PIAS (protein inhibitors of activated STATs) proteins were first discovered in yeast-two-hybrid screens as interacting molecules with ►STAT transcription factors. The mammalian family consists of the founding member PIAS3, which was described as a repressor of STAT3, and three additional members, PIAS1, PIASy (also known as PIAS4), and PIASx (also known as

PIAS2 with α and β splice variants, termed ARIP3 (androgen receptor-interacting protein 3) and Miz1 (*Msx*-interacting zinc finger), respectively. Despite their name, PIAS proteins do not selectively inhibit STAT-mediated ►cytokine signaling (Fig. 1), but function as transcriptional coregulators in diverse pathways either by activating or repressing gene expression [1–4]. PIAS proteins have been shown to interact with a broad range of transcription factors unrelated to STATs, such as p53, c-Jun, Smad, β -catenin/lymphoid enhancer factor 1, NF- κ B p65, nuclear hormone receptors, and other proteins. Recently, PIAS proteins have been identified as ►SUMO E3 ligases, which catalyze the covalent addition of small ubiquitin-like modifier (SUMO) to different target proteins acting in these pathways.

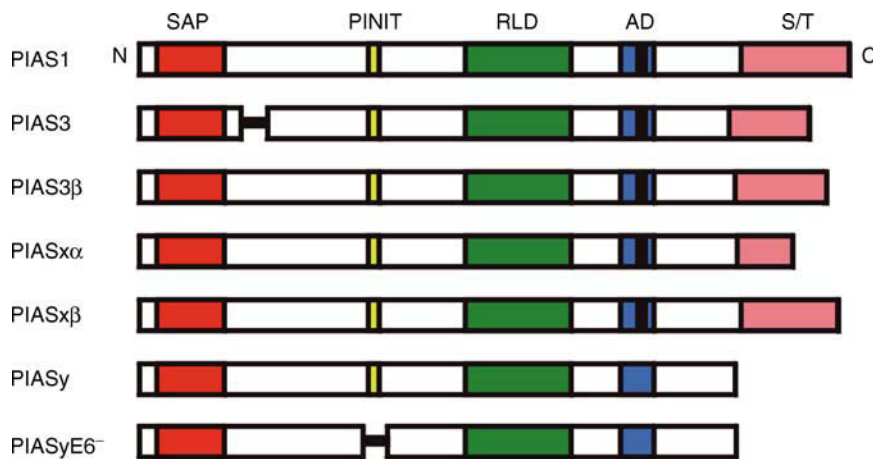
Domain Structure and Function

With the exception of a variable carboxy-terminal region, the mammalian PIAS proteins all share a high degree of sequence homology and a common domain structure (Fig. 2).

The overall sequence identity at the amino acid level is more than 40%. PIAS1 with a length of 651 amino acid residues is the largest member, whereas PIASy lacking the C-terminal serine/threonine-rich domain is the smallest. In the amino-terminus of all PIAS proteins a SAP domain is located which contains an LXXLL amino acid motif (where X denotes any amino acid residue). The acronym refers to three defining members of the class of SAP-containing proteins, namely scaffold-attachment factor (SAF), apoptotic chromatin-condensation inducer in the nucleus (ACINUS), and PIAS, and has been suggested to confer binding to chromatin structures. The SAP domain of PIASy and PIAS1 has been shown to bind synthetic nonspecific (A + T)-rich DNA sequences in



PIAS Proteins. Figure 1 Reporter gene assay with an interferon- γ -driven artificial promoter demonstrating the inhibitory transcriptional activity of increasing amounts of PIAS on STAT1 signal transduction.



PIAS Proteins. Figure 2 Domain structure of PIAS proteins. For abbreviation see text.

vitro, suggesting that it targets PIAS proteins to the nuclear matrix. The PINIT motif is present in all PIAS proteins except the splice variant PIASyE6⁻ which lacks exon 6. Mutation in the PINIT motif results in disrupted nuclear localization of PIAS3, suggesting a role in nuclear retention. Another conserved domain is the RING-finger-like domain (RLD), which resembles the zinc-binding RING fingers found in a subclass of ubiquitin E3 ligases, except for a different spacing of the zinc-coordinating residues. And indeed, the RLD domain has been shown to confer E3 ligase activity for SUMO modification. The carboxy-terminus of PIAS proteins contains a highly acidic domain (AD) with a putative SUMO-interaction motif (SIM; except for PIASy, in which the SIM is missing) and a serine/threonine-rich domain (S/T).

Basic Characteristics

PIAS proteins have been described as adapter molecules that function as E3-like ligase in enhancing the interaction between the SUMO-conjugating enzyme Ubc9 and the respective substrates. PIAS proteins facilitate the formation of an isopeptide bond between the C-terminus of SUMO with the ε-amino group of a lysine residue in the target protein. SUMOylation has been suggested to regulate a variety of cellular processes, such as modulation of transcriptional activity, targeting of proteins to subnuclear structures, and protein stability. PIAS might repress or stimulate gene expression, depending on the target gene and the transcriptional cofactors involved. The molecular mechanisms of PIAS-mediate gene regulation include inhibition of DNA-binding of transcription factors as well as recruiting of coregulators, such as histone deacetylases, p300 or, CPB [cyclic-AMP-responsive-element binding (CREB)-protein]. Sequestering transcription factors in subnuclear structures has been proposed as another mechanism for PIAS action. The

role of SUMOylation in these processes is currently unclear and needs further attention. PIAS1-knockout mice show increased protection against pathogenic infection, but are otherwise viable. PIASy-deficient mice display no obvious phenotype, suggesting a redundancy with other members of the family of PIAS proteins.

Drugs

Specific antagonists of PIAS proteins are not available. The engagement of PIAS proteins in different signal pathways suggests that inhibition of PIAS might cause pleiotropic effects. Due to the promiscuous actions of PIAS, the pharmacological reactions resulting from blockade of PIAS functions are currently far from being predictable. Moreover, the response may differ depending on which member of the PIAS family is preferentially targeted.

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PIC1 (PML Interacting Protein 1)

► SUMOylation

Picornaviruses

Picornaviruses are small, nonenveloped RNA viruses. Members of this family include rhino- and enteroviruses, which are responsible for a variety of human diseases (viral respiratory infection, viral meningitis, myocarditis, pericarditis, encephalitis, chronic meningoencephalitis, herpangina, otitis media, neonatal enteroviral disease, and acute exacerbations of asthma).

►Antiviral Drugs

Picrotoxin

Picrotoxin is a mixture of picrotin (non-toxic) and picrotoxinin, which occurs in the seeds of the Asiatic climber *Anamirta cocculus* (levent berry, cockles). It is a non-competitive antagonist at the γ -aminobutyric acid_A (GABA_A) receptor.

►GABA_A Receptor

PI3-Kinase

Phosphatidylinositol 3-kinase.

PIP

►Phosphatidylinositol Phosphate

PIP₂

►Phosphatidylinositol 4,5-bisphosphate

Pituitary Adenylyl Cyclase-Activating Polypeptide

Pituitary Adenylyl Cyclase-activating Polypeptide (PACAP) is a 38-amino acid peptide (PACAP-38), which is widely expressed in the central nervous system. PACAP is most abundant in the hypothalamus. It is also found in the gastrointestinal tract, the adrenal gland and in testis. Its central nervous system functions are ill-defined. In the periphery, PACAP has been shown to stimulate catecholamine secretion from the adrenal medulla and to regulate secretion from the pancreas. Three G-protein coupled receptors have been shown to respond to PACAP, PAC₁ (PACAP type I) specifically binds PACAP, VPAC₁ and VPAC₂ also bind vasoactive intestinal peptide (VIP). Activation of PACAP receptors results in a G_s-mediated activation of adenylyl cyclase.

PKA

Protein kinase A (PKA) is a cyclic AMP-dependent protein kinase, a member of a family of protein kinases that are activated by binding of cAMP to their two regulatory subunits, which results in the release of two active catalytic subunits. Targets of PKA include L-type calcium channels (the relevant subunit and site of phosphorylation is still uncertain), phospholamban (the regulator of the sarcoplasmic calcium ATPase, SERCA) and key enzymes of glucose and lipid metabolism.

►Adenylyl Cyclases

PKB/Akt

►Akt ►Insulin Receptor

PKC

►Protein Kinase C

PKD2

PKD2, also called polycystin 2, is a TRP-related protein defective in human autosomal polycystic kidney disease, the most common life-threatening genetic disease. PKD2 appears to be a cation channel in the plasma membrane, although there is evidence that it is an intracellular Ca^{2+} release channel. Mammalian homologs include “polycystin-like” (PCL).

► TRP Channels

Placebo Effect

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Synonyms

Placebo response

Definition

The placebo effect is the reduction of a symptom, or a change in a physiological parameter, when an inert treatment (the placebo) is administered to a subject who is told that it is an active drug with specific pharmacological properties. The placebo effect, so far considered a nuisance in clinical research when a new treatment has to be tested, has now become a target of scientific investigation to better understand the physiological and neurobiological mechanisms that link a complex mental activity to different functions of the body. Usually, in clinical research the term placebo effect refers to any improvement in the condition of a group of subjects that has received a placebo treatment, thus it represents a group effect. Conversely, the term placebo response refers to the change in an individual caused by a placebo manipulation. However, these two terms are sometimes used interchangeably. It is important to realize that there is not a single placebo effect but many, which occur through different mechanisms in different conditions, systems and diseases [1, 2, 3].

Basic Mechanisms

Methodological Considerations

The identification of a placebo effect is not easy and its study is full of drawbacks and pitfalls. In fact, the effect which follows the administration of a placebo can be due

to many factors, such as spontaneous remission, i.e. natural history (natural course of a disease or a symptom), regression to the mean (statistical phenomenon which assumes that individuals tend to receive their initial symptom assessment when the symptom is near its greatest intensity, and that their symptom level is likely to be lower when they return for a second assessment), symptom detection ambiguity and biases. All these phenomena need to be ruled out by means of control groups. The possibility of spontaneous remission can be discarded by means of a no-treatment group, which gives us information about the natural course of a symptom. Regression to the mean, can be controlled by using an experimental model in healthy volunteers. Symptom detection ambiguity and biases can be avoided by using objective physiological measurements. It is also important to rule out the possible effects of co-interventions. For example, the mechanical insertion of a needle for the injection of an inert substance may per se induce analgesia, thus leading to erroneous interpretations.

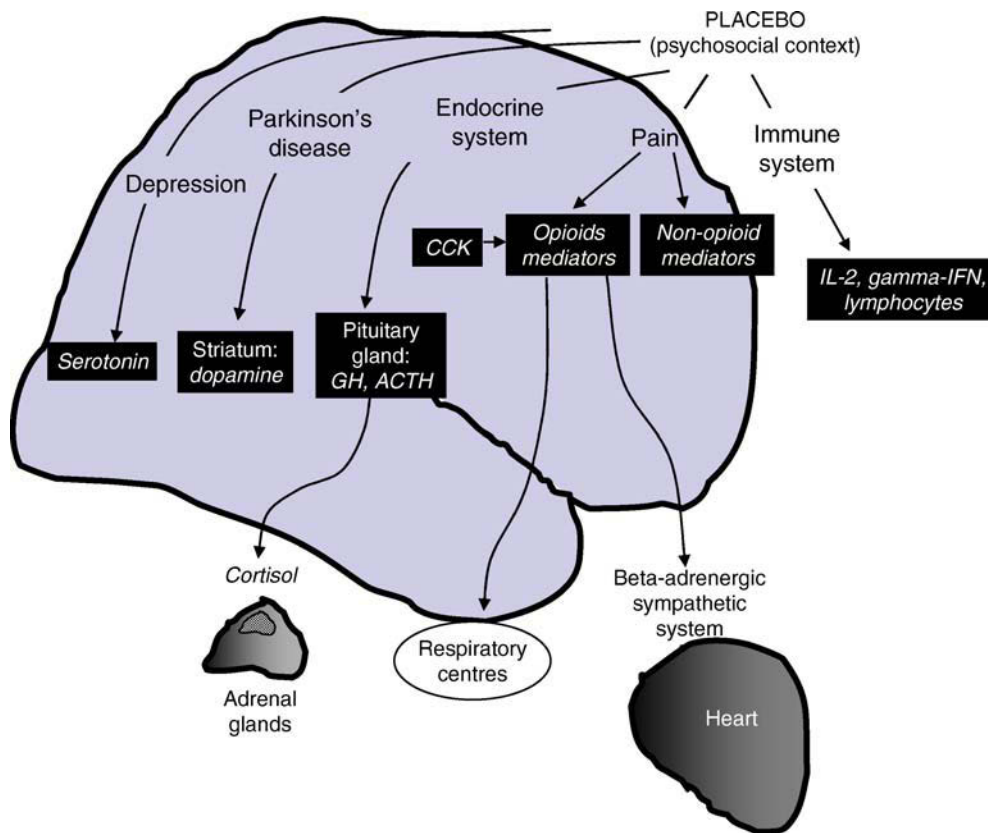
When all these phenomena are ruled out and the correct methodological approach is used, striking placebo effects can be detected which are mediated by psychophysiological mechanisms worthy of scientific inquiry [4, 5]. Therefore, it is this psychological component that represents the real placebo effect.

Psychological Mechanisms

The placebo effect is basically a context effect, whereby the psychosocial context (e.g. the therapist's words, the sight of complex machines, and other sensory inputs) around the medical intervention plays a crucial role. Today we know that the context may produce a therapeutic effect through at least two mechanisms: conscious anticipatory processes and unconscious conditioning mechanisms. In the first case, expectation (anticipation of an event; according to expectation theories, expecting an outcome affects the outcome itself) and anticipation of clinical benefit has sometimes been shown to induce a real clinical improvement. In the second case, contextual cues (e.g. colour and shape of a pill) may act as a conditioned stimulus that, after repeated associations with an unconditioned stimulus (the active pharmacological agent contained in the pill), are capable alone of inducing a clinical improvement. In the case of pain and Parkinson's disease, it has been shown that expectations play a crucial role, even though a ►conditioning procedure is performed.

Neurobiology of Placebo Analgesia

The neural mechanisms underlying the placebo effect are only partially understood and most of our knowledge comes from pain, although recently Parkinson's disease, immune and endocrine responses, and depression have emerged as interesting models (Fig. 1). In each of these



Placebo Effect. Figure 1 Cascade of events that may occur during a placebo procedure in different systems and diseases. In pain, both opioid and non-opioid mediators can be released through the activation of a descending inhibitory network. The respiratory centres may be inhibited by opioid mechanisms as well. The beta-adrenergic sympathetic system is also inhibited during placebo analgesia, although the underlying mechanism is not known (reduction of the pain itself and/or direct action of endogenous opioids). Cholecystikinin (CCK) counteracts the effects of the endogenous opioids, thus antagonizing placebo analgesia, and is also a mediator of the hyperalgesic nocebo effect. Placebos may also affect hormone secretion, like growth hormone (GH), adrenocorticotrope hormone (ACTH) and cortisol, as well as some immune mediators, such as IL-2, gamma-IFN and lymphocytes. In Parkinson's disease, placebos induce dopamine release in the striatum, whereas in depression they affect the same brain regions that are affected by serotonin re-uptake inhibitors, thus suggesting the involvement of serotonin in the placebo effect in depression.

conditions, different mechanisms seem to take place, so that we cannot talk of a single placebo effect but many.

As to pain and analgesia, in the studies so far performed there is a general agreement that the **▶endogenous opioid peptide** systems play an important role in some circumstances. There are several lines of evidence indicating that placebo analgesia is mediated by a descending pain-modulating circuit which uses endogenous opioids as neuromodulators. This evidence comes from a combination of both imaging and pharmacological studies. In fact, by using **▶positron emission tomography (PET)**, it was found that the very same regions of the brain in the cerebral cortex and in the brainstem are affected by both a placebo and the opioid agonist remifentanyl, thus indicating a related mechanism in placebo-induced and opioid-induced analgesia [5]. In particular, the administration of a placebo induces the activation of the rostral

anterior cingulate cortex (rACC), the orbitofrontal cortex (OrbC) and the brainstem. Moreover, there is a significant covariation in activity between the rACC and the lower pons/medulla at the level of the rostral ventromedial medulla (RVM), and a subsignificant covariation between the rACC and the periaqueductal grey (PAG), thus suggesting that the descending rACC/PAG/RVM pain-modulating circuit is involved in placebo analgesia. In another study with **▶functional magnetic resonance imaging (fMRI)**, it was shown that placebo administration produces a decrease of activity in many regions involved in pain transmission, such as the thalamus and the insula.

The studies with PET and fMRI tell us that placebo analgesia and opioid analgesia share a common neural mechanism and that pain transmission is inhibited by placebos, but they do not allow to conclude that the placebo-activated descending network is an opioid

network. In support of the involvement of endogenous opioids in this descending circuit there are several pharmacological studies which show that placebo analgesia is antagonized by the opioid antagonist naloxone. In addition, it has been shown that the endogenous opioid systems have a somatotopic organization, since local placebo analgesic responses in different parts of the body can be blocked selectively by naloxone. A recent PET study used in vivo receptor binding to show that placebos induce the activation of μ opioid receptors in different brain areas, like the dorsolateral prefrontal cortex, nucleus accumbens, insula and rACC.

The placebo-activated endogenous opioids do not act only on pain transmission, but on the respiratory centres as well, since a naloxone-reversible placebo respiratory depressant effect has been described. Likewise, a reduction of **►beta-adrenergic system** activity, which is blocked by naloxone, has been found during placebo analgesia. These findings indicate that the placebo-activated opioid systems have a broad range of action, influencing pain, respiration and the autonomic nervous system, though it is not known whether they act only through a descending modulating network. The placebo-activated endogenous opioids have also been shown to interact with endogenous substances that are involved in pain transmission. In fact, on the basis of the anti-opioid action of **►cholecystokinin** (CCK), CCK-antagonists have been shown to enhance placebo analgesia, thus suggesting that the placebo-activated opioid systems are counteracted by CCK during a placebo procedure.

It is important to point out that some types of placebo analgesia appear to be insensitive to naloxone, thus suggesting that neuromodulators other than opioids can be involved in some circumstances. For example, if a placebo is given after repeated administrations (pre-conditioning) of the non-opioid painkiller ketorolac, the placebo analgesic response is not blocked by naloxone.

Parkinson's Disease

The release of endogenous substances following a placebo procedure is a phenomenon which is not confined to the field of pain, but it is also present in motor disorders, such as **►Parkinson's disease**. As occurs with pain, in this case patients are given an inert substance (placebo) and are told that it is an anti-parkinsonian drug that produces an improvement in their motor performance. A study used PET in order to assess the competition between endogenous dopamine and ^{11}C -raclopride for D_2/D_3 receptors, a method that allows identification of endogenous dopamine release [4]. This study shows that placebo-induced expectation of motor improvement activates endogenous dopamine in the striatum of Parkinsonian patients. As this occurs in both the dorsal and ventral striatum, a region involved in reward, it has been argued that the

expectation-induced release of dopamine in Parkinson's disease is related to reward mechanisms.

Placebo administration in Parkinson patients affects the activity of the neurons in the subthalamic nucleus, a brain region belonging to the basal ganglia circuitry and whose activity is increased in Parkinson's disease. Verbal suggestions of motor improvement during a placebo procedure are capable of reducing the firing rate and abolishing bursting activity of subthalamic nucleus neurons, and these effects are related to clinical improvement [1].

Immune and Endocrine Responses

Placebo responses in both the immune and endocrine system can be evoked by pharmacological pre-conditioning. In fact, after repeated administrations of drugs, if the drug is replaced with a placebo, immune or hormonal responses can be evoked that are similar to those obtained by the previously administered drug. For example, immunosuppressive placebo responses can be induced in humans by repeated administration of cyclosporine A (unconditioned stimulus) associated to a flavoured drink (conditioned stimulus), as assessed by interleukin-2 (IL-2) and interferon- γ (IFN γ) mRNA expression, in vitro release of IL-2 and IFN γ , and lymphocyte proliferation. Likewise, if a placebo is given after repeated administrations of sumatriptan, a serotonin agonist of the 5-HT $_{1B/1D}$ receptors that stimulates **►growth hormone** (GH) and inhibits cortisol (**►glucocorticoids**) secretion, a placebo GH increase and a placebo cortisol decrease can be found. These studies support a conditioning mechanism in both immunosuppressive and hormonal placebo responses.

Depression

Depressed patients who receive a placebo treatment show both electrical and metabolic changes in the brain. In the first case, placebos induce electroencephalographic changes in the prefrontal cortex of patients with major depression, particularly in the right hemisphere. In the second case, changes in brain glucose metabolism were measured by using PET in subjects with unipolar depression. Placebo treatments are associated with metabolic increases in the prefrontal, anterior cingulate, premotor, parietal, posterior insula, and posterior cingulate cortex, and metabolic decreases in the subgenual cingulate cortex, parahippocampus and thalamus. Interestingly, these regions are also affected by the selective serotonin re-uptake inhibitor, fluoxetine, a result that suggests a possible role for serotonin in placebo-induced antidepressant effects.

Nocebo Effect

The nocebo effect, or response, is a placebo effect in the opposite direction. For example, administration of an inert substance along with verbal suggestions of pain

increase may induce a hyperalgesic effect. In this case, anticipatory anxiety may play a fundamental role. Nocebo hyperalgesia has been found to be blocked by proglumide, a non-specific CCK-A/CCK-B receptor antagonist. This suggests that expectation-induced hyperalgesia is mediated, at least in part, by CCK. These effects of proglumide are not antagonized by naloxone, thus endogenous opioids are not involved. Since CCK plays a role in anxiety and negative expectations themselves are anxiogenic, proglumide is likely to act on a CCK-dependent increase of anxiety and pain during the verbally induced negative expectations. Although, mainly due to ethical constraints, the nocebo effect has not been investigated in detail, as has been done for the placebo effect, it shows the powerful effect of the top-down modulation of pain. In other words, cognitive and emotional factors can modulate pain perception in opposite directions.

Pharmacological Relevance Implications for Clinical Trials

According to the classical methodology of clinical trials, any drug must be compared with a placebo in order to assess its effectiveness. If the group that takes the drug shows a larger clinical improvement than the group that takes the placebo, the drug is considered to be effective. However, in light of the recent advances in placebo research, some caution is necessary in the interpretation of some clinical trials. In fact, by considering the complex cascade of biochemical events induced by placebo administration, any drug that is tested in a clinical trial may interfere with these placebo/expectation-activated mechanisms, thus confounding the interpretation of the outcome of a clinical trial. As we have no a priori knowledge of which substances act on placebo-activated endogenous opioids, dopamine, serotonin – and indeed almost all drugs might interfere with these neurotransmitters – one way to eliminate this possible pharmacological interference is to make the placebo-activated biochemical pathways ‘silent’. This can be achieved by the hidden administration of drugs.

Hidden Administration of Drugs

It is possible to eliminate the placebo (psychosocial) component and analyze the pharmacodynamic effects of a treatment, free of any psychological contamination, by administering drugs covertly. In this way, the cascade of biochemical events triggered by a placebo procedure can be eliminated. To eliminate the psychosocial context in which a treatment is given, and thus the placebo component of the treatment, the patient is not made aware that a medical therapy is being carried out. To make this possible, drugs are administered through hidden infusions by machines. A hidden drug infusion can be performed through a computer-controlled infusion pump that is pre-programmed to deliver the drug at the desired time. It is crucial that the patient does

not know that any drug is being injected, so that he or she does not expect anything. The computer-controlled infusion pump can deliver a drug automatically, without a doctor or nurse in the room, and without the patient being aware that a treatment has been started [3].

The analysis of different treatments, either pharmacological or not, in different conditions has shown that an open (expected) therapy, that is carried out in full view of the patient, is more effective than a hidden one (unexpected). Whereas the hidden injection represents the real pharmacodynamic effect of the drug, free of any psychological contamination, the open injection represents the sum of the pharmacodynamic effect plus the psychological component of the treatment. The latter can be considered to represent the placebo component of the therapy, even though it cannot be called placebo effect, as no placebo has been given. It is important to realize that, by using hidden administration of drugs, it is possible to study the placebo effect without the administration of any placebo.

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Placebo Response

► Placebo Effect

Placenta Growth Factor

Placenta growth factor is a vascular endothelial growth factor (VEGF)-related molecule, which binds to VEGF-R1.

► Angiogenesis and Vascular Morphogenesis

Planar Cell Polarity Pathway

Synonyms

PCP Pathway

Definition

The Wnt/non- β -catenin-dependent signaling pathway in *Drosophila melanogaster* comprised of genes affecting the transverse orientation of cells in an epithelial sheet. Like Wnt/ β -catenin-dependent signaling it relies on Fz receptors and Dvl, but unlike Wnt/ β -catenin-dependent signaling it involves activation of RhoA (and possibly JNK) to mediate its effects. It is not entirely certain that PCP signaling involves a Wnt ligand at all. A molecularly conserved pathway in vertebrate cells controls cell morphology and convergent-extension movements during development.

► Wnt Signaling

Plaque

An atherosclerotic lesion consisting of a fibrotic cap surrounding a lipid-rich core. The lesion is the site of inflammation, lipid accumulation, and cell death. Also known as an atheroma.

► Atherosclerosis

Plasma Binding

In vitro parameter reflecting on the bound drug in blood or in plasma.

► Pharmacokinetics

Plasma ChE

► Cholinesterases

Plasma Lipid Transfer Proteins

► Lipid Transfer Proteins

Plasmid

Plasmids are extrachromosomal, mainly circular genetic elements that reproduce autonomously within the bacterial cell. Their sizes vary from 1 to over 1,000 kbp. Plasmids are not essential for growth but carry genes that may confer selective growth advantage in specific environments. Examples are genes controlling the production of toxins, virulence factors or enzymes allowing the catabolism of unusual substrates. R plasmids (resistance plasmids) confer resistance to antibiotics and various other inhibitors of growth, e.g. heavy metals.

► Microbial Resistance to Drugs

α_2 -Plasmin Inhibitor

The α_2 -plasmin inhibitor, a single-chain glycoprotein (70 kDa), forms rapidly an equimolar complex with plasmin, where the enzyme loses its activity. It is synthesized by the liver and secreted into the blood circulation, where its concentration is 1 μ M.

► Fibrinolytics

Plasmin(ogen)

Plasmin, a serine protease (83 kDa), can degrade fibrin, and its degradation products (FDP) are soluble in the blood. Plasmin is formed from its proenzyme (zymogen, precursor), plasminogen (92 kDa), synthesized by the liver, and secreted into the blood circulation, where its concentration is $\sim 2 \mu$ M. Plasminogen is converted to plasmin by plasminogen activators (serine proteases).

► Fibrinolytics

Platelet-activating Factor

Synonyms

PAF

Definition

A ubiquitous phospholipid mediator (acetyl-glycerol-ether-phosphorylcholine) in inflammation. Among others, it stimulates platelet aggregation and causes swelling, smooth contraction or dilation and neutrophil activation.

► Bronchial Asthma

Platelet Aggregation Inhibitors

► Antiplatelet Drugs

Platelet-derived Growth Factor Receptor

The platelet-derived growth factor receptor (PDGFR) is a protein found on the surface of endothelial cells to which platelet-derived growth factor (PDGF) binds. When PDGF attaches to PDGFR, it activates the enzyme tyrosine kinase, triggering reactions that cause angiogenesis. PDGFR is found at abnormally high levels on the surface of many types of pericytes in cancer environment, which may divide excessively in the presence of PDGF.

► Growth Factors
► Targeted Cancer Therapy

Platelet Inhibitors

► Antiplatelet Drugs

Platelets

Platelets are the formed elements of the blood which participate in hemostasis. Platelets are enucleated, discoid fragments which arise from mature megakaryocytes in the bone marrow. Under normal circumstances, platelets do not adhere to endothelial surfaces of blood vessels. However, platelets can adhere to damaged areas of blood vessels and become activated in such a way that they can also bind fibrinogen.

► Antiplatelet Drugs

Platinum Complexes

Certain platinum-containing compounds are capable of crosslinking DNA and kill cells by similar pathways to alkylating chemotherapeutic agents.

► Alkylating Agents

Pleckstrin Homology Domain

Pleckstrin homology domain (PH-domain) was first identified at the amino and carboxyl termini of a haematopoietic protein called pleckstrin. PH-domain, a protein region of approximately 120 amino acids, by binding to phosphatidylinositol lipids of the biological membranes induces the translocation of the PH-domain containing protein to membrane compartment. Various PH-domains possess specificities for phosphoinositides phosphorylated at different sites within the inositol ring.

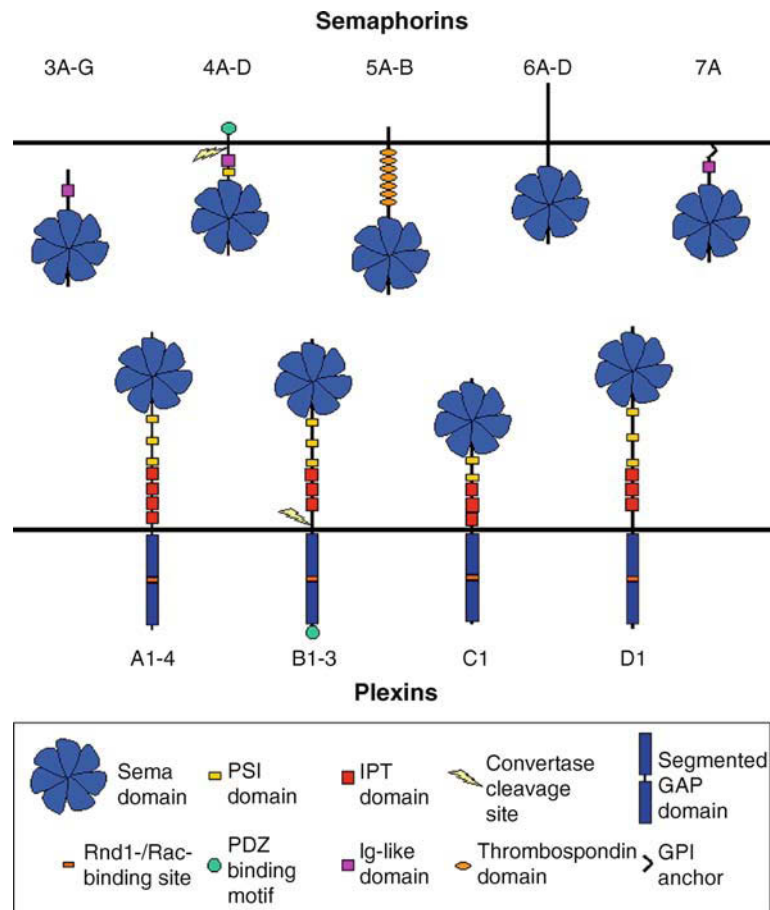
► Phospholipid Glossary

Plexins

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Definition

Plexins comprise a family of transmembrane proteins that serve as receptors for ► [semaphorins](#). On the basis



Plexins. Figure 1 Mammalian plexins and their semaphorin ligands. The mammalian plexins are grouped into four subfamilies (A–D). The mammalian semaphorin family is divided into five classes (3–7). Both plexins and semaphorins are characterized by the presence of an extracellular Sema domain which contains a seven-blade β -propeller. In addition, all Plexins share a highly conserved intracellular moiety that carries a segmented GAP-domain. Additional domains include PSI (plexin, semaphorin, integrin)-domains and IPT (Ig-like, plexins and transcription factors)-domains. The plexins of the B-family have a PDZ-binding-motif at their C-terminus. Some B-Plexins exhibit a protease cleavage site. While class-3-semaphorins are secreted, class 4–6 are transmembrane proteins. Class-7-semaphorins are attached to the plasma membrane by a GPI-anchor. Class-5-semaphorins are characterized by seven thrombospondin domains.

of homology they are grouped into four subfamilies (A–D). Semaphorins are a large family of secreted and membrane-associated proteins. In the mammalian system, five structurally different classes of semaphorins (3–7) have been found (Fig. 1). While the semaphorin–plexin system was initially discovered as a regulator of **axonal guidance** in the developing nervous system, it has become clear that its physiological and pathophysiological relevance is much larger including organogenesis, angiogenesis, the immune system as well as cancer.

Basic Characteristics

Both plexins and semaphorins are structurally characterized by the presence of an extracellular **sema domain** which contains a seven-blade β -propeller. The

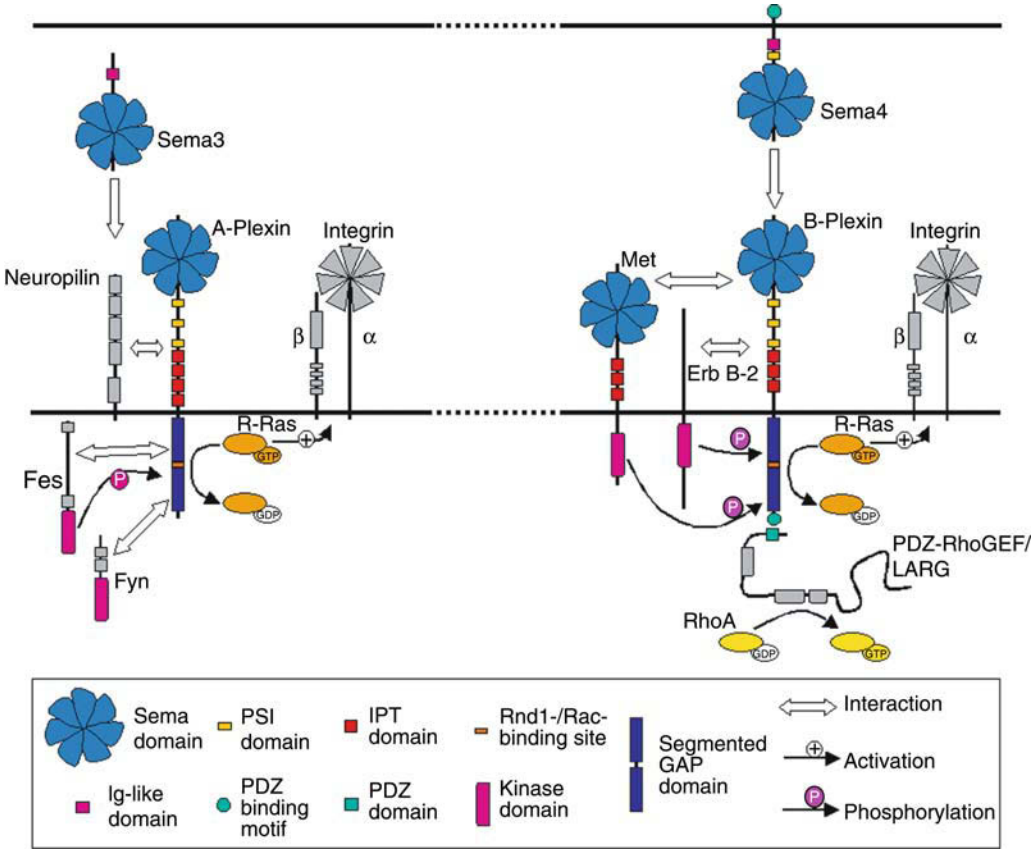
cytoplasmic moieties of plexins (~600 amino acids) are highly conserved and do not show homology to any other known protein. Since semaphorins are found both in a membrane-bound form as well as in a soluble form, plexins can interact with semaphorins on other cells or with semaphorins in solution. The interaction between semaphorins and plexins is mediated by the Sema domains (Table 1). However, the class 3 semaphorins do not bind directly to plexins but to **neuropilins**, which interact with plexins of the A-family. In this complex, neuropilins are responsible for binding the class 3 semaphorins while the A-plexins are required for transferring the signal over the plasma membrane. In contrast to the A-plexins, B-, C- and D-plexins bind semaphorins directly via their Sema domain.

Plexins. Table 1 Plexins and their respective semaphorin ligands

Receptor	Ligand
Plexin-A1/neuropilin-1	Sema3A
Plexin-A1/neuropilin-2	Sema3F
Plexin-A1	Sema6D
Plexin-A2/neuropilin-1	Sema3A
Plexin-A3/neuropilin-1	Sema3A
Plexin-A3/neuropilin-2	Sema3F
Plexin-A4/neuropilin-1	Sema3A
Plexin-A4/neuropilin-2	Sema3F
Plexin-A4	Sema6A, Sema6B
Plexin-B1	Sema4D
Plexin-B2	Sema4C, Sema4D
Plexin-B3	Sema5A
Plexin-C1	Sema7A
Plexin-D1	Sema3E

Activation of plexins results in the induction of a variety of signalling pathways. All plexins contain a GTPase-activating protein (GAP) domain within their cytoplasmic domain which has been shown to promote the guanine nucleotide exchange of R-Ras. Inhibition of R-Ras has been suggested to result in the inhibition of integrin signalling. In addition, plexins of the A-family have been shown to interact with cytosolic tyrosine kinases like Fes or Fyn as well as with the GTP-bound form of the small GTPase Rac. Plexins of the B-family carry at their C-terminus a PDZ-binding motif which mediates a stable interaction with the Rho guanine nucleotide exchange factors (RhoGEFs) LARG and PDZ-RhoGEF. Activation of B-plexins results in the activation of RhoA via this pathway (Fig. 2). There is evidence that receptor tyrosine kinases like c-Met and c-ErbB-2 directly interact with B-plexins and are involved in plexin-B-dependent signalling.

Semaphorins and plexins were first described to be involved in the guidance of axonal growth cones during



Plexins. Figure 2 Intracellular signalling processes induced via plexins. Both the activation of A-Plexins (left) and B-Plexins (right) lead to an inactivation of R-Ras via their conserved cytoplasmic RasGAP domains, thereby inhibiting the activation of integrins by RasGTP. Binding of class-3-semaphorins to neuropilin activates A-plexins, which in turn results in an activation of the cytosolic tyrosine kinases Fes and Fyn. Binding of class-4-semaphorins to B-plexins stimulates the kinase activity of the receptor tyrosine kinases Met and ErbB-2. In addition, activation of B-plexins leads to an activation of RhoA via the RhoGEF (Rho guanine nucleotide exchange factors) activity of PDZ-RhoGEF or LARG.

development. *Sema3A* (formerly *collapsin-1*) was in fact one of the first repulsive axonal guidance factors described. Various semaphorins have been shown to induce axonal growth cone repulsion by activation of plexins. The axonal growth cone retraction induced by semaphorins is mediated by small GTPases. Both plexin A- and plexin B-family members are able to sequester GTP-bound Rac which normally induces the formation of cell extensions (lamellipodia). In addition, activation of the A- and B-plexin families results in an inhibition of R-Ras via their R-Ras-GAP domain. Since GTP-bound R-Ras activates integrins, the activation of plexins via the inhibition of R-Ras activity results in integrin inhibition which may promote axonal growth cone collapse and neurite retraction. Moreover, B-family plexins activate the Rho/Rho-kinase-mediated signalling pathway which results in the stimulation of actomyosin-based contractility to induce cell retraction and axonal growth cone collapse. Besides the repulsive activity of semaphorins, there is also evidence that semaphorins regulate axon branching and in some cases may have attractive activity.

There is increasing evidence that class 3 semaphorins play a role in the regulation of guidance and remodelling of the vascular system. Similar to the situation in the nervous system, the semaphorin–plexin system also appears to be involved in the guidance of specialized endothelial cells at the tip of navigating vascular sprouts during vascular development. In addition to a repulsive effect on endothelial cells in developing blood vessels, class 3 semaphorins have also been shown to be required for the fusion of vessels to create large blood vessels.

Various semaphorins and plexins are found to be expressed in the immune system. *Sema4D* (=CD100), e.g. is expressed by T-cells as well as by activated B-cells and antigen-presenting cells. In the immune system, *Sema4D* appears to primarily act via the non-plexin receptor CD72. *Sema4D* stimulates the activation and maturation of dendritic cells. There is evidence that *Sema4D* in immune cells may itself be involved in signal transduction processes, and it has been shown to associate with the protein tyrosine phosphatase CD45. One of the first indications for the importance of semaphorins in the immune system came from studies on viral semaphorins. *Vaccinia virus* e.g. carries a gene closely resembling the mammalian *Sema7A* which is expressed by activated T-cells and which binds to plexin-C1 on dendritic cells or monocytes. Expression of the viral *Sema7A* homologue has been shown to suppress migration of host antigen-presenting dendritic cells and monocytes to various infected cells.

The semaphorin–plexin system has been shown to be involved both in tumour suppression and tumour progression. The genes encoding *Sema3B* and *Sema3F*

are candidate tumour suppressors often found to be inactivated in small cell-lung cancer. Class 3 semaphorins may be repulsive for endothelial cells thereby inhibiting angiogenesis and appear to inhibit integrin activation resulting in impaired attachment and metastasis. Other class 3 semaphorins like *Sema3C* and *Sema3E* as well as *Sema4D*, *Sema5C* and *Sema6A/B* have been suggested to contribute to tumorigenesis or to tumour progression. Several semaphorins and plexins have been found to be overexpressed in human tumours.

Pharmacological Intervention

The semaphorin–plexin system has been shown to play multiple roles in the nervous system, the immune system, during the development of various organ systems as well as in the progression of certain tumours. Further studies are needed to more clearly define the role of individual semaphorins and plexins in various physiological and pathophysiological processes. There is some evidence that interfering with semaphorin–plexin signalling could improve regeneration after neuronal injury, and recent data indicate that a *Sema3A* inhibitor improves regenerative responses and functional recovery in a rat model of spinal cord injury. Blockade of the action of some semaphorins should have interesting immunomodulatory effects as shown by the action of various viral semaphorins or in mice lacking *Sema4D*. Finally, the activation or inhibition of defined semaphorin–plexin systems may be a useful approach for the treatment of certain cancers.

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Polyamines

Polyamines are low molecular weight aliphatic nonprotein nitrogenous bases. Putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm) are the most common natural polyamines. They are synthesized by decarboxylation of ornithine and methionine. At physiological pH, these polyamines can be considered as organic polycations, Put²⁺, Cad²⁺, Spd³⁺ and Spm³⁺. Polyamines are essential for many growth-related processes such as DNA replication, RNA synthesis and translation, as well as for block of some ion channels. Putrescine and spermidine are ubiquitous in living organisms, while spermine is rare or even nonexistent in prokaryotes. In neurons they exert an activity-dependent block of the channel pore of Ca²⁺-permeable AMPARs.

- Potassium Channels
- Inward Rectifier K⁺ Channels
- Ionotropic Glutamate Receptors

Polyenes

- Antifungal Drugs

Polyethylene Glycol

Polyethylene glycol is a water-soluble polymer that can be covalently attached to certain molecules, e.g., filgrastim. Addition of polyethylene glycol does not change the identity, purity, or biological activities of filgrastim, but plasma clearance is decreased and the circulating half-life of filgrastim is increased.

- Hematopoietic Growth Factors

Polymorphism

Polymorphism describes a naturally occurring variation in the DNA sequence among individuals which leads to the occurrence of two or more allelic forms of a gene.

Examples of a polymorphism include single nucleotide substitutions, insertions and deletions of nucleotides, and repetitive sequences. While most polymorphisms are harmless and part of normal human genetic variations, studies have established links between certain gene polymorphisms and metabolic alterations or human diseases.

- Pharmacogenomics

Polypeptide Chain Binding Proteins

- Chaperones

POMC

- Prepro-opiomelanocortin

Poor Metabolizer Phenotype

P

Synonyms

PM phenotype

Definition

A drug metabolism phenotype characterized by much slower biotransformation rate compared to the average population who exhibits an extensive metabolizer (EM) phenotype. Usually the PM phenotype is genetically determined by the lack of a functional gene copy of the drug metabolizing enzyme that catalyzes the respective metabolic step. For example, PMs for debrisoquine or sparteine carry two null-alleles of *CYP2D6*, the enzyme that oxidizes both drugs but they are also genetically determined PMs of many antidepressants or beta-blockers which are also substrates of this enzyme. Additional phenotypes are possible, for example due to alleles with partially reduced activity (intermediate metabolizer, IM) or due to alleles with enhanced activity (ultrarapid metabolizer, UM). In contrast to the permanent genetically determined PM

phenotype, a transient PM phenotype can also be caused by enzyme inhibition (also referred to as *phenocopying*).

- P450 Mono-oxygenase System
- Pharmacogenetics

Population Pharmacokinetics

Population pharmacokinetics is the application of pharmacokinetic and statistical methods to sparse data to derive a pharmacokinetic profile of central tendency.

- Pharmacokinetics

Positron Emission Tomography

Positron emission tomography (PET) is an imaging technique that relies on the emission of positrons from radionucleotides tagged to an injectable compound of interest. Each positron emitted by the radioisotope collides with an electron to emit two photons at 180° from each other. The photons are detected and the data processed so that the source of the photons can be identified and an image generated showing the anatomical localization of the compound of interest.

Post-translational Modification

Post-translational modification (PTM) is the enzymatic processing of a protein into the biologically active form following release from the ribosome. This includes removal and/or derivatization of specific amino acid residues, proteolytic cleavage, loss of signal sequences, and formation of disulfide crosslinks. Other common posttranslational modifications are phosphorylation, glycosylation, acetylation, hydroxylation, myristylation, isoprenylation, and many other reactions. All amino acid side chains except those of alanine, glycine, isoleucine, leucine, and valine can be modified.

- Proteomics
- Ubiquitin/Proteasome

Potassium Channels

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Synonyms

K⁺ channels

Definition

Potassium channels are a diverse and ubiquitous family of membrane proteins present in both excitable and nonexcitable cells that selectively conduct K⁺ ions across the cell membrane along its electrochemical gradient at a rate of 10⁶–10⁸ ions/s.

Basic Characteristics

Under physiological conditions, the concentration of K⁺ ions inside the cell is around 25-fold greater than that outside the cell membrane. An outward current is generated due to the efflux of K⁺ ions by the opening of K⁺ channels that bring membrane potential close to the resting state (repolarization) of the cells. K⁺ channels set the resting membrane potential, shorten the action potential, terminate periods of intense activity, and determine the interspike intervals during repetitive firing [1]. Activators of K⁺ channels tend to stabilize cellular excitability or dampen the effectiveness of excitatory inputs whereas blockers tend to have the opposite effects. In addition to controlling cellular excitability, K⁺ channels can regulate fluid and electrolyte transport and cell proliferation.

About 78 human genes encoding a variety of K⁺ channels and auxiliary subunits have been identified (Fig. 1; Table 1). While the K⁺ channels are diverse, they share with a unique conducting pore highly selective for K⁺ ions. The K⁺ channels are tetramers composed of four α subunits that form the conducting pore. On the basis of primary amino acid sequence of α subunit, K⁺ channels can be classified into three major families (Fig. 2).

Six Transmembrane One-Pore Channels

Molecular biology studies have identified a loop containing 20–25 amino acid residues between S5 and S6 (or M1 and M2, Fig. 2) forming the pore. The G(Y/F) G motif located in the pore represents the K⁺-selectivity signature, which is common to all K⁺ channels. The external entry to the channel pore and its adjacent residues constitute binding sites for toxins and blockers. The internal vestibule of the pore and the adjacent residues in S5 and S6 contribute to binding sites for compounds such as 4-aminopyridine and quinidine. The S4–S5 linker lies close to the permeation pathway and is required for

a Voltage-gated K⁺ channel

	Name	Common name	Gene	Chromosome	Tissue expression	Genetic disorders
	K _V 11.1	Herg	<i>KCNH2</i>	7q35	B, H	
	K _V 11.3	erg-3	<i>KCNH7</i>	2q24.2	B, H	
	K _V 11.2	erg-2	<i>KCNH6</i>	17q23.3	B, H	
	Kv10.1	eag-1	<i>KCNH1</i>	1q32	B	
	K _V 10.2	eag-2	<i>KCNH5</i>	14q24	B	
	K _V 12.1	Elk-1	<i>KCNH8</i>	3p24.3	Nerve tissue	
	K _V 12.3	Elk-3	<i>KCNH14</i>	17q21	B	
	K _V 12.2	Elk-2	<i>KCNH3</i>	12q13	B	
	K _V 7.4		<i>KCNQ4</i>	1p34	Haircell, inner ear	Deafness
	K _V 7.5		<i>KCNQ5</i>	6q14	B, Skm	
	K _V 7.2		<i>KCNQ2</i>	20q13	B, neuron	BFNC
	K _V 7.3		<i>KCNQ3</i>	8q24	B, neuron	BFNC
	K _V 7.1	KVLQT1	<i>KCNQ1</i>	11p15.5	H, Co, K, L, PI	LQT1 (JLNS)
	K _V 4.2		<i>KCND2</i>	7q31-32	B	
	K _V 4.3		<i>KCND3</i>	1p13.2	B, H	
	K _V 4.1		<i>KCND1</i>	Xp11.23-11.3	H, B, L, K, Li, PI	
	K _V 1.1		<i>KCNA1</i>	12p13	B, H, R, Panc	EA
	K _V 1.2		<i>KCNA2</i>	1p13	B, H, Panc	
	K _V 1.3		<i>KCNA3</i>	1p21-p13.3	Lym, B, L, Th, S	
	K _V 1.4		<i>KCNA4</i>	11q13.4-14.1	B, H, Panc	
	K _V 1.7		<i>KCNA7</i>	19q13.3	H, Panc, Skm	
	K _V 1.6		<i>KCNA6</i>	12p13	B	
	K _V 1.5		<i>KCNA5</i>	12p13	B, H, K, L, Skm	
	K _V 1.8		<i>KCNA10</i>	1p13	K, H, aorta	
	K _V 3.1		<i>KCNC1</i>	11p15	B, M, Lym	
	K _V 3.4		<i>KCNC4</i>	1p21	B, Skm	
	K _V 3.3		<i>KCNC3</i>	19q13.3-13.4	B, L	
	K _V 3.2		<i>KCNC2</i>	19q13.3-13.4	B	
	K _V 6.1	KH2	<i>KCNG1</i>	20q13	B	
	K _V 6.2		<i>KCNG2</i>	18q22-18q23	H	
	K _V 6.3		<i>KCNG3</i>	2p21	B, K, L, Panc	
	K _V 9.2		<i>KCNS2</i>	8q22	B	
	K _V 9.3		<i>KCNS3</i>	2p24	L, B, artery	
	K _V 9.1		<i>KCNS1</i>	20q12	B	
	K _V 2.1		<i>KCNB1</i>	20q13.2	B, H, K, Skm, R	
	K _V 2.2		<i>KCNB2</i>	8q12	Sm	
	K _V 8.1		<i>KCNB3</i>	8q22.3-24.1	B	
	K _V 5.1	KH1	<i>KCNF1</i>	2p25	B	

b Ca²⁺-activated K⁺ channel

KCa2.2	SKCa2	KCNN2	5q21.2-q22.1	B, H, L
KCa2.3	SKCa3	KCNN3	1q21.3	B, Adrg, Tcells
KCa2.1	SKCa1	KCNN1	19p13.1	B, H
KCa3.1	IKCa1	KCNN4	19q13.2	Lym, Col, Pros, PI
KCa4.1	Slack	KCNMB2	9q34	ubiquitous
KCa4.2	Slo2	KCNT2	1q31	
KCa1.1	Slo	KCNMA1	10q23.1	B, Sm, Panc
KCa5.1	Slo3	KCNMA3	8p11	testis

Potassium Channels. Figure 1 (Continued)

inactivation. The S4 segment containing 5–7 positive charges at approximately every third position serves as voltage-sensor governing the channel opening.

Two Transmembrane One-Pore Channels

The inward rectifier K⁺ channels (Kir) represent this family of channels that conduct K⁺ ions preferentially in the inward direction than outward. The occlusion of internal vestibule of the pore by Mg²⁺ and polyamines contribute to this inward rectification. These channels

are tetrameric, although a more complex octameric arrangement has been described for ATP-sensitive K⁺ (K_{ATP}) channels derived from four inward rectifiers contributing to the ion conducting pore and four regulatory sulfonylurea receptor subunits.

Four Transmembrane Two-Pore Channels

This family of K⁺ channels, with >50 distinct gene members, typically contains four putative transmembrane and two pore domains. The G(Y/F)G motif is

c Two-Pore K⁺ channel

	Name	Common name	Gene	Chromosome	Tissue expression	Genetic disorders
	K _{2P} 12.1	THIK2	<i>KCNK12</i>	2p22-2p23	B, K	
	K _{2P} 13.1	THIK1	<i>KCNK13</i>	14q24.1-24.3	Ubiquitous	
	K _{2P} 3.1	TASK1	<i>KCNK3</i>	2p23	H, B, Panc, PI	
	K _{2P} 9.1	TASK9	<i>KCNK9</i>	8q24	B, K, Li, L	
	K _{2P} 15.1	TASK5	<i>KCNK15</i>	20q12	Adrg, Panc	
	K _{2P} 1.1	TWIK1	<i>KCNK1</i>	1q42-q43	B, K, H	
	K _{2P} 6.1	TWIK2	<i>KCNK6</i>	19q13.1	K	
	K _{2P} 7.1	KCNK7	<i>KCNK7</i>	11q13	B	
	K _{2P} 10.1	TREK2	<i>KCNK10</i>	14q31	B	
	K _{2P} 2.1	TREK1	<i>KCNK2</i>	1q41	B	
	K _{2P} 4.1	TRAAK	<i>KCNK4</i>	11q13	B, Spin, R	
	K _{2P} 16.1	TALK1	<i>KCNK16</i>	6p21	Panc	
	K _{2P} 17.1	TALK2	<i>KCNK17</i>	6p21	Panc, PI, L, H, Li	
	K _{2P} 5.1	TASK2	<i>KCNK5</i>	6p21	B, L	
	K _{2P} 18.1	TRESK	<i>KCNK18</i>	10q26.11	Spinal cord	

d Inward-rectifying K⁺ channel

	Kir4.1	<i>KCNJ10</i>	1q22	Glial	
	Kir4.2	<i>KCNJ15</i>	21q22.2	K, L, B	
	Kir1.1	<i>KCNJ1</i>	11q24	K, Panc	Bartter's
	Kir6.1	<i>KCNJ8</i>	12p11.23	ubiquitous	PHHI
	Kir6.2	<i>KCNJ11</i>	11p15.1	ubiquitous	
	Kir2.1	<i>KCNJ2</i>	17q23	H, B, Sm/Skm, K	Andersen's
	Kir2.2	<i>KCNJ12</i>	17p11.1-11.2	H	
	Kir2.3	<i>KCNJ4</i>	22q13.1	H, B, Skm	
	Kir2.4	<i>KCNJ14</i>	19q13	B, R	
	Kir3.2	<i>KCNJ6</i>	21q22.1-22.2	Panc, cerebellum	
	Kir3.4	<i>KCNJ5</i>	11q24	H, Panc	
	Kir3.3	<i>KCNJ9</i>	1q21-23	H, B, Skm	
	Kir3.1	<i>KCNJ3</i>	2q24.1	H, cerebellum	
	Kir5.1	<i>KCNJ16</i>	17q25	B, periphery	
	Kir7.1	<i>KCNJ13</i>	2q37	GI, K, B	

Potassium Channels. Figure 1 Human potassium channel genes: localization and diseases Human K⁺ channel genes are sorted by similarity of amino acid sequence. The dendrogram was generated using Pileup program of the Wisconsin Sequence Analysis Package (Genetics Computer Group (GCG), Madison, Wisconsin). Abbreviations: Adrg, adrenal gland; Andersen's, Andersen's syndrome; B, brain; Bartter's, Bartter's syndrome; BEC, brain-specific eag-like channel; BFNC, Benign familial neonatal convulsions; BK_{Ca}, large-conductance Ca²⁺-activated K⁺ channel; Co, cochlea; EA, Episodic ataxia/myokymia syndrome; EAG, *ether-a-go-go* gene encoded K⁺ channel; GI, gastrointestinal; H, heart; HEAG, human *ether-a-go-go*; hSK, human small-conductance Ca²⁺-activated; IK_{Ca}, intermediate-conductance Ca²⁺-activated; JLNS, Jervell and Lange-Nielsen syndrome; K, kidney; Kv, voltage-gated; Kir, inward-rectifier K⁺ channel; L, lung; Li, liver; LQT, long-QT syndrome; Lym, lymphocyte; M, muscle; Pan, pancreatic islet; PHHI, Persistent hyperinsulinemic hypoglycemia of infancy; PI, placenta; Pros, prostate; R, retina; Skm, skeletal muscle; Sm, smooth muscle; Spin, spinal cord; TASK, TWIK-related acid-sensitive K⁺ channel; TASK, TWIK-related acid-sensitive K⁺ channel; TRAAK, TWIK-related arachidonic acid-stimulated K⁺ channel; TWIK, two-pore weak inward rectifier.

preserved in the first pore loop, but this motif is replaced by GFG or GLG in the second pore loop. The two-pore K⁺ channel family plays an important role in conducting K⁺ leak currents that regulate cellular excitability by shaping the duration, frequency, and amplitude of action potentials through their influence over the resting membrane potential [2].

K⁺ Channelopathies

Genetically Linked Diseases

Genetic linkage analyses have identified inherited human disorders due to abnormal function of K⁺

channel subunits. Such naturally occurring mutations result in losses or changes in K⁺ channel function [3].

Cardiac diseases: In the heart, multiple K⁺ channels regulate cardiac excitability and determine cardiac action potential duration. Both KvLQT1 and **MinK** have wide distribution in different tissues. These two subunits coassemble to form a slowly activating delayed rectifier K⁺ channel (IK_S) that participates in repolarization of cardiac action potential, and regulates transepithelial K⁺ ion secretion in the inner ear. Mutations in either KvLQT1 or MinK have been linked to cardiac arrhythmia as in the dominant **Long-QT**

Potassium Channels. Table 1 Human K⁺ channel auxiliary subunits: genes, localization, function, and disorders

Name	Gene	Chromosome	Tissue Expression	Association with α Subunits	Function(s)	Disorders
Kv β 1	<i>KCNAB1</i>	3q26.1	Brain, heart	Kv1.4; Kv1.5	Inactivation	
Kv β 2	<i>KCNAB2</i>	1p36.3	Brain, heart	Kv1.4	Inactivation	
Kv β 3	<i>KCNAB3</i>	17p13.1	Brain	Kv1.5	Inactivation	
MINK	<i>KCNE1</i>	21q22.1–22.2	Heart, kidney, uterus	KvLQT1	Partner for cardiac IK _S channel	LQT5
MiRP1	<i>KCNE2</i>	21q22.1	Heart, skeletal muscle	hERG	Partner to form the cardiac IK _R channel	LQT6
MiRP2	<i>KCNE3</i>	11q13–q14	Small intestine, colon, kidney	KvLQT1; KvLQT4; hERG	(i) Coassemble with KvLQT1 to form cAMP-activated K ⁺ channel in intestine; (ii) suppress KvLQT4 currents; (iii) suppress hERG currents	
SUR1	<i>SUR1</i>	11p15.1	Pancreas, neurons, skeletal muscle	Kir6.2	Coassemble to form K _{ATP} channels	PHHI
SUR2A/SUR2B	<i>SUR2</i>	12p12.1	SUR2A: Heart, skeletal muscle SUR2B: Brain, smooth muscle, liver	Kir6.2 or Kir6.1	Coassemble to form K _{ATP} channels	
BK _{Ca} ⁻ β 1	<i>KCNMB1</i>	5q34	Smooth muscle, cochlea, pancreas	BK _{Ca}	Alter Ca ²⁺ and voltage-dependency	
BK _{Ca} ⁻ β 2	<i>KCNMB2</i>	3q26.2–q27	Kidney, heart, uterus	BK _{Ca}	Inactivation; iberiotoxin-resistant	
BK _{Ca} ⁻ β 3	<i>KCNMB3</i>	3q26.3–q27.1	Spleen, testis, placenta, pancreas	BK _{Ca}		
BK _{Ca} ⁻ β 4	<i>KCNMB4</i>	12q14.1–q15	Brain, heart, kidney	BK _{Ca}	Iberiotoxin-, charybdotoxin-resistant	

syndromes (LQT) or to recessive LQT, in which cardiac arrhythmia is associated with congenital deafness. Another cardiac rapid delayed rectifier K⁺ channel (IK_R), in contrast to IK_S, is composed of *hERG* and MiRP1, and is responsible for repolarization of cardiac action potential. Aberrant function of hERG/MiRP1 resulting from mutations in either subunit has been identified from families associated with LQT. Mutations in *KCNJ2* encoding Kir2.1 lead to ►**Andersen's syndrome**.

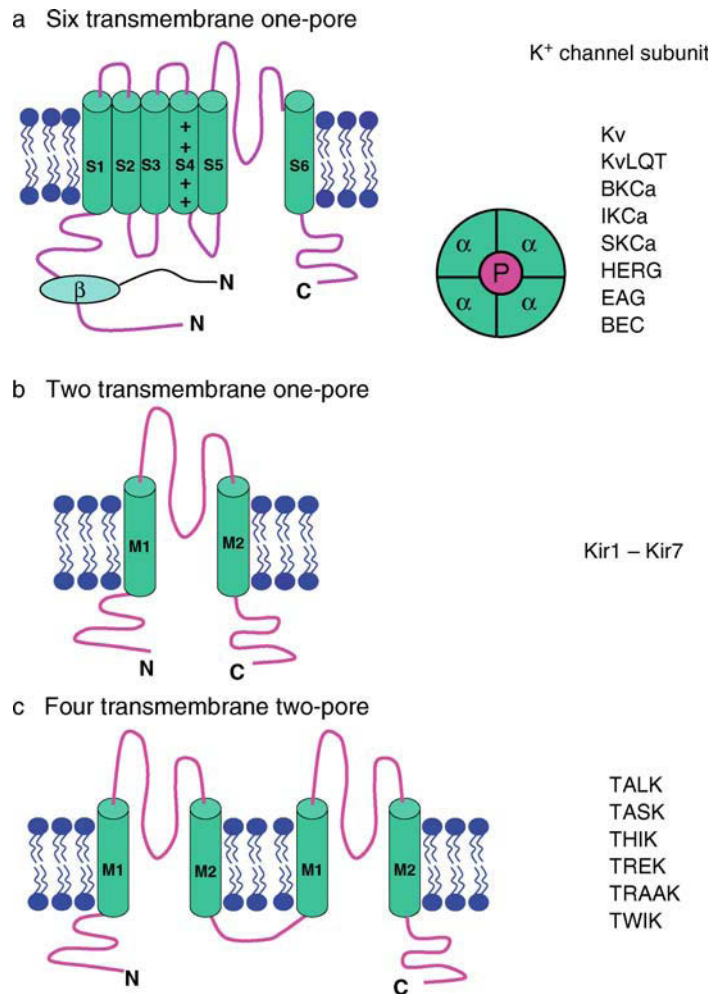
CNS diseases: In the CNS, the mutations in *KCNAL1* (Kv1.1) impair the capacity of the affected neurons to repolarize effectively following an action potential and are linked to the type 1 ►**episodic ataxia/myokymia** (EA1). KCNQ3 and KCNQ2 or KCNQ5 constitute diverse ►**M-channels** that play important roles in determining the excitability threshold, firing properties, and responsiveness of neurons to synaptic inputs. Mutations in both KCNQ2 and KCNQ3 subunits have been identified in families associated with ►**benign familial neonatal convulsions** [4].

Deafness: KCNQ4 is expressed in vestibular system, brain, and cochlea sensory hair cells. KCNQ4 has been

mapped to human chromosome 1p34, in which DFNA2 (The DFNA2 locus for autosomal dominant nonsyndromic hearing impairment, the one of over 30 loci for dominant deafness, is located in chromosome 1p34. In DFNA2 locus, at least mutations in two genes, GJB3 encoding connexions to form gap junction and KCNQ4 encoding K⁺ channels have been linked to progressive hearing loss.) is located. Loss of KCNQ4 function due to deletion or mutation contributes to progressive hearing loss [4].

Renal diseases: Mutations in *KCNJ1* disrupt the function of Kir1.1 in apical renal outer medulla of the kidney. The loss of tubular K⁺ channel function and impaired K⁺ flux could prevent apical membrane potassium recycling and lead to antenatal ►**Bartter's syndrome**.

Metabolic diseases: In the pancreatic β -cells, K_{ATP} channel derived from ►*SUR1* and Kir6.2, links cellular metabolism to electrical activity and regulates insulin secretion. Mutations in SUR1 and Kir6.2 that result in loss of K_{ATP} channel function have been identified in families with ►**familial persistent hyperinsulinemic hypoglycemia of infancy** (PHHI).



Potassium Channels. Figure 2 Schematic representation of the structural classification of K⁺ channel subunits. (a) Six-transmembrane one-pore subunits. This represents a class of the K⁺ channels composed of four subunits each containing six transmembrane segments (S1–S6) and a conducting pore (P) between S5 and S6 with a voltage sensor (positive charge of amino acid) located at S4. Some of the voltage-gated K⁺ channels include an auxiliary β -subunit (Kv β), which is a cytoplasmic protein with binding site located at the N-terminus of the α -subunit. The inset shows the general assembly of K⁺ channels. The homotetrameric K⁺ channel consists of four identical subunits while different α -subunits form heterotetrameric K⁺ channels. (b) Two-transmembrane one-pore subunits. The inward rectifier K⁺ channel belongs to a superfamily of channels with four subunits each containing two-transmembrane segments (M1 and M2) with a P-loop in between. (c) Four-transmembrane two-pore subunits. This represents a class of K⁺ channel that has four transmembranes with two P-loops. This figure is adapted from [3].

Acquired Aberrant K⁺ Channel Function

In addition to naturally occurring mutations in K⁺ channels leading to various human disorders, acquired dysfunction of K⁺ channels induced by drugs or diseases can also occur [3]. Blockade of hERG channel by certain H1 antagonists, antipsychotics, tricyclic antidepressants, antibiotics, and anti-emetic agents contributes to the drug-induced LQT leading to polymorphic ventricular dysrhythmia, the **torsade de pointes**. H1 receptor antagonists such as loratadine and rupatadine can also induce cardiac arrhythmia by blocking Kv1.5. Up- or down-regulation of K⁺ channel

gene expression has been shown to be involved in cardiac hypertrophy, atrial fibrillation, apoptosis, oncogenesis, and Alzheimer's disease. In neuromuscular junction, inhibition of delayed rectifier K⁺ channels (Kv1.1 and Kv1.6) by autoantibodies is the underlying mechanism leading to muscle twitching observed in **Isaacs' syndrome**, an acquired neuromyotonia.

Drugs

Concurrent with the progress in our understanding of molecular diversity, structure, and function of K⁺ channels, and their role in genetically linked and acquired

diseases, interest in the discovery and development of selective modulators of various classes of K^+ channels has evolved [5].

Voltage-Gated K^+ Channels

Kv1.3: The Kv1.3 channel plays a critical role in controlling Ca^{2+} influx that regulates proliferation in human T lymphocytes. Blocking Kv1.3 inhibits activated T cell proliferation, thereby making this channel an attractive target for immunosuppressant agents. In addition to correolide, other known Kv1.3 blockers include H-37, WIN-17317-3, CP-339818, UK-78282, ShK (L5)-amide (SL5), and PAP1 (Table 2).

Kv1.5: In human atria, the Kv1.5 represents the ultrarapid delayed rectifier that contributes to the repolarization in the early phase of cardiac action potential. Selective blockers of Kv1.5 channels could be potentially beneficial in the treatment of atrial fibrillation because blocking Kv1.5 could delay repolarization and prolong refractoriness selectively in cardiac myocytes. Examples for Kv1.5 blockers include AVE0118, S9947, and analogs of diphenyl phosphine oxide (DPO).

hERG: hERG/MiRP is the major target for the class III antiarrhythmic agents. Novel and selective agents include dofetilide, ibutilide, and azimilide that block

Potassium Channels. Table 2 Potassium channel openers^a

Channel family	Therapeutic Indications	Compounds
ATP-sensitive K^+ channels (K_{ATP})		
Cardiac Mitochondria K_{ATP} (SUR2A/Kir6.2)	Myocardial ischemia	BMS-180448
		BMS-191095
		Diazoxide
SUR2B/Kir6.2	Angina	Nicorandil, JTV-506
(SUR2B/Kir6.1?)	Hypertension	Pinacidil,
(SUR2B splice variant/Kir6.2)		Aprikalim (RP 52891),
		Bimakalim (EMD52692),
		Cromakalim, Celikalim,
		Emakalim, NIP121
		RO 316930, RWJ 29009,
		SDZ PCO 400,
		Rimakalim (HOE234),
		Symakalim (EMD 57283),
		YM-099, YM-934
	Airway hyperactivity	SDZ-217-744
	Alopecia	P1075, Minoxidil
	Bladder overactivity	ZM244085, ZD6169,
		WAY151616,
		ZD0947, A-278637, A-151892, A-251179
	Erectile dysfunction	PNU-83757
Ca^{2+} -activated K^+ channels (K_{Ca})		
Large-conductance (BK_{Ca})	Cerebral ischemia	BMS-204352
	Vascular/nonvascular disorders	NS1608, NS-4, NS1609 (also Ca^{2+} channel blocker)
	Antipsychotic	
	Urinary incontinence	NS-8
	Pollakisuria	
Intermediate (IK_{Ca})-, Small-conductance (SK_{Ca})	Vascular disorder	1-EBIO, chlorzoxazone, zoxazolamine
	Cystic fibrosis	
M-channels		
KCNQ2/KCNQ3	Epilepsy	retigabine (also GABA _A agonist), BMS-204352

^aAdapted from Shieh et al. [3].

hERG, prolong cardiac action potential, and represent useful agents for the treatment of arrhythmias (Table 2).

KvLQT1: KvLQT1/MinK has been considered a promising avenue for class III antiarrhythmic approach. As noted above, most known class III antiarrhythmic drugs block hERG/MiRP and lead to prolongation of cardiac action potentials. However, hERG/MiRP blockade typically causes excessive prolongation of action potentials at slow heart rates, whereas at higher rates, blockade is much less effective. This so-called reverse use-dependent action can lead to life-threatening arrhythmias. Thus, selective KvLQT1/MinK blockers may be regarded as a more promising approach with reduced proarrhythmic potential. Chromanol 293B is a prototypical inhibitor of KvLQT1/MinK. Other blockers include HMR-1556 and L-73582.

Other KCNQ-derived channels: The M-channel has emerged as an attractive target for cognitive disorders. Several neurotransmitter release enhancers initially shown to be cognition enhancers such as linopirdine, DMP-543, and XE-991 are M-channel blockers. Mutations in KCNQ2 and KCNQ3 linking to benign familial neonatal convulsions suggest that M-channel openers may have as potential as antiepileptic agents. The antiepileptic agent retigabine has been shown to activate KCNQ2/KCNQ3 channels, indicating that M-channel activation may be another mode of action for anticonvulsant drugs.

K_{ATP} Channels

As noted previously, SUR1/Kir6.2 constitutes the pancreatic K_{ATP} channel. The molecular composition of the cardiac/skeletal muscle K_{ATP} channel is SUR2A/Kir6.2, whereas SUR2B/Kir6.2 is thought to form the predominant K_{ATP} channel in smooth muscle cells. Like sarcolemmal K_{ATP} channels, mitochondria K_{ATP} channel also couples the energy metabolism to cellular activities although its molecular composition remains to be elucidated.

SUR1/Kir6.2: Glibenclamide and glipizide that block pancreatic K_{ATP} channels have been used for the treatment of type II diabetes. New class of insulin secretagogues includes repaglinide and nateglinide, which improve insulin secretion, action and reduce carbohydrate absorption.

Mitochondria K_{ATP} channels: Cardiac K_{ATP} channel opening has a role in myocardial preconditioning, a paradoxical form of cardioprotection wherein brief ischemic episodes can protect the heart from subsequent lethal ischemic injury. Openers including BMS-180448 and BMS-191095 have been reported to possess preferential cardioprotective effects over vasorelaxant effects by activating mitochondria K_{ATP} channels.

SUR2B/Kir6.2: Efforts have also focused on the development of selective K_{ATP} channel openers for vascular and nonvascular indications including angina, airway hyperactivity, bladder overactivity, and erectile

dysfunction (Tables 2 and 3). Examples of structurally diverse smooth muscle active K_{ATP} channel openers include ZD-6169, WAY-133537, A-278637, A-151892, and A-251179.

Ca²⁺-Activated K⁺ Channels

This subfamily including the large- (BK_{Ca}), intermediate- (IK_{Ca}), and small-conductance (SK_{Ca}) Ca²⁺-activated K⁺ channels are activated by increases in intracellular free Ca²⁺ concentration. The opening of IK_{Ca} and SK_{Ca} channels are less voltage-dependent, whereas the activation of BK_{Ca} channel has steep voltage sensitivity.

BK_{Ca}: The diversity of BK_{Ca} channels can be attributed to the assembly of pore-forming α subunit together with four different auxiliary subunits (β 1– β 4). BMS-204352 has been identified as a BK_{Ca} channel opener for the treatment of acute ischemic stroke although it has also been shown as an M-channel activator. Therapeutic applications for channel openers include epilepsy, bladder overactivity, asthma, hypertension, and psychosis. Other known BK_{Ca} channel openers include NS-8, NS-1619, NS-4, and certain aminoazaindole analogs.

IK_{Ca}: The IK_{Ca} channel corresponds to the *Gardos* channel described in red blood cells. Blockers of IK_{Ca} channels have been suggested for the treatment of sickle cell anemia, diarrhea, and rheumatoid arthritis. Blockers of IK_{Ca} channels may be used as immunosuppressive agents because these channels are upregulated following antigenic or mitogenic stimulation in T-cells. IK_{Ca} channel blockers include clotrimazole, ICA-15451, and TRAM-34. Openers of IK_{Ca} channels may be therapeutically beneficial in cystic fibrosis and peripheral vascular diseases. Although not highly specific, 1-EBIO (1-ethyl-2-benzimidazolinone) chlorzoxazone and zoxazolamine have been shown to activate IK_{Ca} channels.

SK_{Ca}: The SK_{Ca} channels are responsible for the slow after hyperpolarization and play important roles in determining the firing frequency. Distinct genes are known to encode SK_{Ca}1, SK_{Ca}2, and SK_{Ca}3, in which SK_{Ca}2 and SK_{Ca}3 are highly apamin-sensitive. Overexpression of SK_{Ca}3 can induce abnormal respiratory responses to hypoxic challenge and compromised parturition, suggesting the SK_{Ca}3 as potential target for sleep apnea and for regulating uterine contractions during labor. Other indications for SK_{Ca} channel modulators include myotonic muscular dystrophy, gastrointestinal dysmotilities, memory disorders, and epilepsy. SK_{Ca} channel blockers include dequalinium analogs and more potent agents such as UCL-1684 and UCL-1530.

Two-Pore K⁺ Channels

The two-pore K⁺ channels are thought to function as background channels involved in the resting membrane

Potassium Channels. Table 3 Potassium channel blockers^a

Channel family	Therapeutic Indications	Compounds
Voltage-gated K ⁺ channels		
Kv1.3	Immunosuppressant	Correolide, WIN-17317-3, CP-339318, UK-78, 282, H-37, PAP-1
Kv1.5 (IK _{UR})	Atrial fibrillation	NIP-142
Kv4.2 (IK _{TO})	Arrhythmia	Flecainide, Clofilium
Kv (other)	Multiple sclerosis	Fampridine (4-aminopyridine)
	Epilepsy	BIIA 0388
hERG/MiRP (IK _R)	Arrhythmia	Dofetilide, Ibutilide
	Atrial fibrillation/flutter	Almokalant, E4031, MK499, Sematilide, Azimilide (NE 10064), D-Sotalol
KvLQT1/MinK (IK _S)	Arrhythmia	Chromanol 293B, HMR1556, E-047/1, L768673, L735821, L364373
Kv1.5, hERG/MiRP, KvLQT1/MinK	Arrhythmia, angina	Ambasilide (LU 47710), Tedisamil
ATP-sensitive K ⁺ channels (K _{ATP})		
SUR1/Kir6.2	Type II diabetes	Tolbutamide, Chlorpropamide, Glibenclamide, Glipzide, Nateglinide, Repaglinide
SUR2A/Kir6.2	Ventricular arrhythmia, Sudden cardiac death	HMR-1883 (Clamikalant), HMR-1098
SUR2B/Kir6.2 (?)	Arrhythmia, diuretics	PNU-37883A, PNU-99963, IMID-4F
Ca ²⁺ -activated K ⁺ channels		
Intermediate-conductance (IK _{Ca})	Sickle cell anemia, Diarrhea, Immunosuppressant Rheumatoid arthritis	Clotrimazole, ICA-15451 ICA-17043 TRAM-34
Small-conductance (SK _{Ca})	Sleep apnea Neuromuscular disorders	Dequalinium, Tubocurarine, UCL-1684, UCL-1530
M-channels		
KCNQ2/KCNQ3	Cognition enhancer Alzheimer's disease	Linopirdine (DUP 996), DMP-543, XE-991

^aAdapted from Shieh et al. [3].

potential regulation. Certain neuroprotective agents such as riluzole and volatile general anesthetics can activate two-pore K⁺ channels, suggesting these channels might be attractive targets for novel neuroprotective and anesthetic agents. The endocannabinoid, anandamide, is a blocker of the TASK channel.

Conclusions

Defects and dysfunction of K⁺ channels have emerged as the molecular basis for a number of diseases. Enhanced knowledge of K⁺ channel structure and function, together with further analysis of genetic- and disease-induced dysregulation of K⁺ channels could undoubtedly improve diagnosis and offer specific candidate genes for the development of appropriate therapies. Technologies to

refine high throughput assay techniques for K⁺ channel modulators should be helpful to identify potent, and more importantly, selective drug candidates.

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Potassium Competitive Acid Blockers

► Proton Pump Inhibitors and Acid Pump Antagonists

PP

► Pancreatic Polypeptide

PPAR

A nuclear receptor that is a key transcription factor in adipocytes. It plays a critical role in the control of adipocyte differentiation and is involved in the regulation of the expression of specific adipokines, including leptin and adiponectin. It has anti-inflammatory actions and is the target of the thiazolidinedione drugs.

- Adipokines
- Peroxisome Proliferator-Activated Receptors (PPARs)

PPCs

Peroxisome Proliferator Chemicals.

PR

Progesterone Receptor.

► Sex Steroid Receptors

Pregnane X Receptor

The pregnane X receptor (PXR) is a promiscuous nuclear receptor, that has evolved to protect the body from toxic chemicals. It is activated by a wide variety of xenobiotics including several drugs like rifampicin, hyperforin (the active ingredient of St. John's wort), clotrimazole and others. PXR heterodimerizes with the retinoid X receptor (RXR) and is also activated by various lipophilic compounds produced by the body such as bile acids and steroids. PXR heterodimerized with RXR stimulates the transcription of cytochrome P450 3A monooxygenases (CYP3A) and other genes involved in the detoxification and elimination of the potentially harmful substances. PXR appears to be the key regulator of CYP3A induction by xenobiotics.

► Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes

Preintegration Complex

The preintegration complex is a complex of retroviral DNA and proteins that translocates from the cytosol into the nucleus prior to integration.

► Gene Therapy

Prenylation

Prenylation is the post-translational addition of 15- or 20-carbon isoprenyl lipids to the C-terminus of proteins. Prenylation is an irreversible modification that anchors proteins to the membrane fraction of cells.

► Lipid Modifications

Prepro-opiomelanocortin

Prepro-opiomelanocortin (POMC) is a huge precursor protein, from which several peptides are generated by

proteolytic cleavage (melanocyte-stimulating hormone, adrenocorticotrophic hormone (ACTH), β -endorphin, methionine enkephalin).

- ▶ Anti Obesity Drugs
- ▶ Opioid System

Primary Hemostasis

Primary hemostasis is the first phase of hemostasis consisting of platelet plug formation at the site of injury. It occurs within seconds and stops blood loss from capillaries, arterioles, and venules. Secondary hemostasis, in contrast, requires several minutes to be complete and involves the formation of fibrin through the coagulation cascade.

- ▶ Antiplatelet Drugs

Primary Hyperparathyroidism

Synonyms

PHPT

Definition

A form of PTH-dependent hypercalcemia caused by enlargement and hyperfunction of one or more parathyroid glands.

- ▶ Ca^{2+} -Sensing Receptor

Prodrug

A prodrug is a drug that is not by itself pharmacologically active but needs metabolic activation by an enzyme. Examples are the cytostatic cyclophosphamide, which is activated by hydroxylation catalyzed by CYP2B6, or HMGCoA reductase inhibitor, lovastatin, which contains

a lactone ring that must be cleaved by carboxylesterases to yield the active free carboxylic acid.

- ▶ P450 Mono-oxygenase System
- ▶ Pharmacokinetics

Progesterone Receptor

- ▶ Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

Progestins

Progestins are derived from the 21-carbon series and contain as a basic structure the pregnane nucleus. In the non-pregnant female they are mainly produced in the ovary.

- ▶ Contraceptives
- ▶ Selective Sex Steroid Receptor Modulators
- ▶ Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

Programmed Cell Death

- ▶ Apoptosis

Prolactin

Prolactin is peptide hormone secreted by the pituitary gland. It acts on prolactin receptors in breast tissue where it stimulates production of casein and lactalbumin. It also acts on the testes and ovaries to inhibit the effects of gonadotrophins. Since the secretion of prolactin is under tonic dopaminergic inhibition by the hypothalamus, dopamine D_2 -receptor antagonists

cause prolactin release through a process of disinhibition, leading to elevated prolactin levels resulting in gynaecomastia, galactorrhoea, anovulation, and impotence.

- ▶ Antipsychotic Drugs
- ▶ JAK-STAT Pathway

Proopiomelanocortin

Proopiomelanocortin (POMC) is the precursor peptide of hormones and neuropeptides expressed in the pituitary and the hypothalamus (adrenocorticotrophic hormone (ACTH), lipotropin, α -melanocyte-stimulating hormone (α MSH), γ MSH, β -endorphin, and others). The main clinical consequences of POMC deficiency are adrenal insufficiency (due to absence of ACTH), red hair pigmentation (due to absence of MSH) and severe early-onset obesity (due to the lack of α MSH).

- ▶ Appetite Control
- ▶ Opioid System
- ▶ Melanocortin

Propionylcholinesterase

- ▶ Cholinesterases

Prostacyclin

- ▶ PGI₂
- ▶ Prostanoids

Prostaglandin H2 Synthase

- ▶ Cyclooxygenases
- ▶ PGHS

Prostaglandins

Prostaglandins are a group of lipid autacoids known as eicosanoids. They are produced from membrane phospholipids and found in almost every tissue and body fluid. They are involved in a number of physiological processes including inflammation, smooth muscle tone and gastrointestinal secretion. In the central nervous system they have been reported to produce both excitation and inhibition of neuronal activity.

- ▶ Prostanoids

Prostanoids

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Synonyms

Prostaglandins; Dinoprostone; ▶ Eicosanoids

Definition

▶ **Prostanoids**, or prostaglandins, are potent mediators of a wide range of physiological actions including pain, inflammation, modulation of smooth muscle tone as well as water and ion transport. Prostaglandins are oxygenated metabolites of the essential fatty acid ▶ **arachidonic acid**. Four of the principal prostaglandins are analogs of the 20 carbon unnatural fatty acid prostanic acid, distinguished by its five carbon “prostane” ring group comprised of carbons five through eight. The fifth prostanoid, thromboxane, has an inserted ether oxygen and thus has a six-member ring structure and is an analog of the unnatural fatty acid thrombanoic acid [1].

Basic Characteristics

Biosynthesis

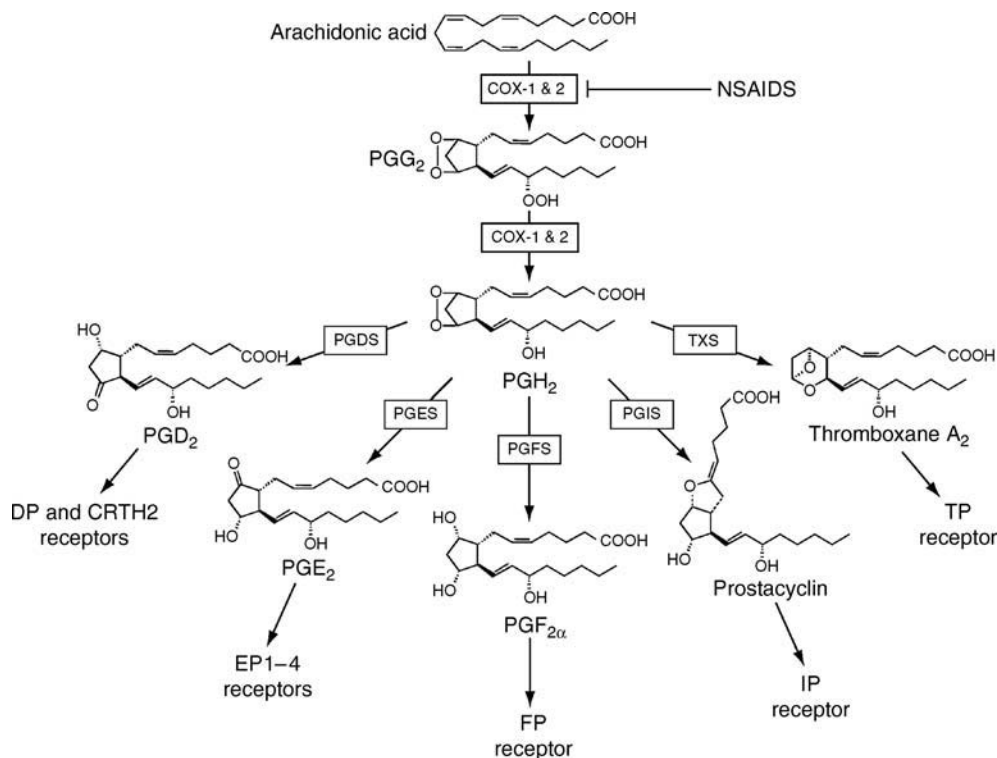
PGs act locally in an autocrine or paracrine fashion in the tissues in which they are synthesized, rather than as circulating hormones, which act at a distant site. For this reason, studies localizing the enzymatic machinery, which synthesize prostaglandins, are informative with respect to the site of PG actions. PG synthesis is

initiated by ►cyclooxygenase (COX) mediated metabolism of the unsaturated 20 carbon fatty acid arachidonic acid to PGG₂/H₂, generating five primary bioactive prostanoids: PGE₂, PGF_{2α}, PGD₂, PGI₂ (prostacyclin), and TXA₂ (thromboxane; Fig. 1). Arachidonic acid is esterified in the lipid bilayer of most cells, and is liberated by the action of specific phospholipases, for example, PLA₂. Mobilization of arachidonic acid by phospholipases is regulated by a number of hormones and signal transduction pathways and represents a critical control point of prostanoid synthesis. Upon liberation, arachidonic acid is rapidly metabolized by a number of enzymatic pathways including the cyclooxygenase pathway. Cyclooxygenase, also known as PGH synthase, catalyzes two sequential reactions, a *bis* oxygenase, or cyclooxygenase, reaction leading to the formation of PGG₂ and a subsequent peroxidase activity at the C15 position leading to the conversion of PGG₂ to PGH₂ [2]. Two isozymes of cyclooxygenase have been identified, designated COX-1 and COX-2, which catalyze the formation of identical products, but have different patterns of expression and differential regulation. Differential blockade of the COX isozymes results in important physiological outcomes, as described below. PGH₂, the immediate product of COX

activity, is an unstable product that spontaneously degrades to other prostaglandin metabolites; however, in vivo, PGH₂ is acted upon by specific PG synthases leading to differential shunting of PGH₂ to one of the five principal prostanoid products. Following their formation, PGs cross the cell membrane where local concentrations of PGs may be modulated by specific transporters including the recently described specific PG transporter (PGT). Although PGT's role is incompletely understood, it has been proposed to facilitate the reuptake of PGs to allow vectorial transport of PGs synthesized in polarized cells.

Receptor Pharmacology

The local action of PGs depends, in part, on activation of a family of specific G-protein coupled receptors (►GPCRs), designated EP for E-prostanoid receptors, FP, DP, IP, and TP receptors, respectively, for the other prostanoids [3]. The EP receptors are unique in that four receptors, designated EP1 through EP4, have been described for PGE₂ each encoded by a distinct gene. A second class of Prostaglandin D receptor designated Chemoattractant Receptor-Homologous molecule expressed on ►Th2 cells (CRTH2) which has no sequence homology to the remaining PG receptors has been



Prostanoids. Figure 1 Biosynthesis of prostaglandins. Arachadonic acid is metabolized by cyclooxygenase-1 or 2 to the unstable endoperoxide PGH₂, the common precursor for the five principle prostaglandins. PGD₂, PGE₂, PGF_{2α}, PGI₂, and thromboxane A₂ are generated by individual prostaglandin synthase enzymes and elicit their biological effects by activating cell surface G-protein coupled receptors.

identified. This receptor has been unofficially designated “DP2.” Each of the other PGs has a single receptor, and taken together, there are nine PG GPCRs, each encoded by distinct genes (Table 1). Alternative mRNA splice variants have been cloned for the EP1, EP3, TP, and FP receptors. In each case, these splice variants generate receptor sequence diversity in the intracellular C-terminal tail of the receptor protein (Fig. 2). Functionally, these splice variants appear to modulate the specificity of G-protein coupling, as well as regulation of receptor desensitization by encoding alternate phosphorylation sites. Pharmacologically, the PG receptors are distinguished by their ligand binding selectivity as well as the signal transduction pathways they activate. In general, a PG receptor may have significant affinity for PGs other than its principal ligand. Moreover, multiple PG receptors are frequently coexpressed in a single cell type or tissue. COX activation and resulting PG production may lead to complex effects in the target tissue by activation of multiple PG receptor subtypes. Thus, a given PG ligand may elicit multiple, and at times apparently opposing, functional effects on a given target tissue. For example, prostaglandin receptors were initially characterized by their actions on smooth muscle, where they may lead to either smooth muscle contraction or relaxation. The vasodilator effects of PGE₂ have long been recognized in both arterial and venous beds. Smooth muscle relaxation by PGE₂ is, however, not uniformly observed, and PGE₂ is a potent constrictor in other smooth muscle beds, including trachea, gastric fundus, and ileum. Importantly, some structural analogs of

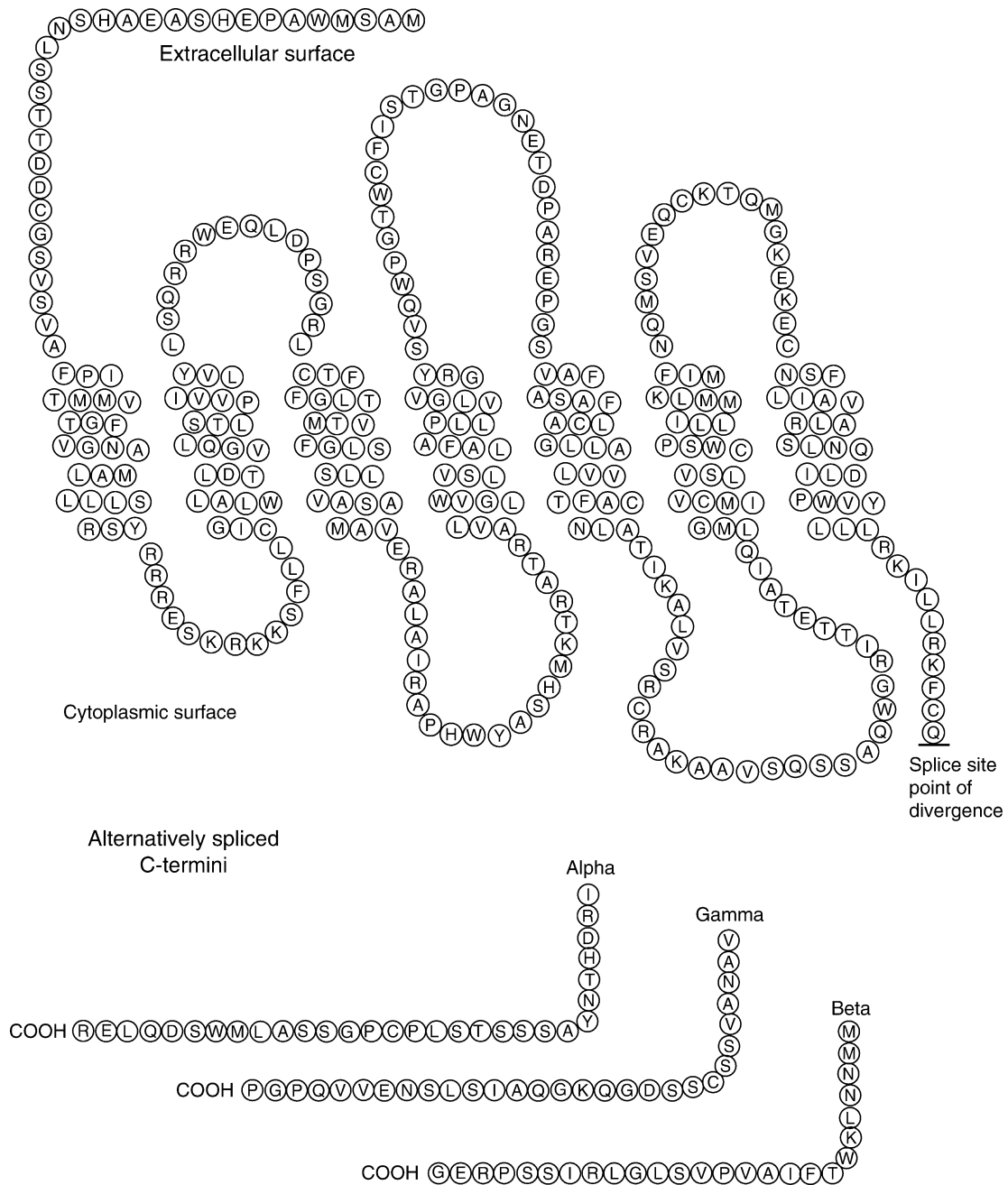
PGE₂ are capable of reproducing the dilator effects of PGE₂, but are inactive on tissues where it is a constrictor. Conversely, analogs that reproduce the constrictor effects of PGE₂ may fail to affect tissues where PGE₂ is a dilator. The EP receptor mRNAs exhibit differential expression in a number of tissues with distinct functional consequences of activating each receptor subtype. Functional antagonism among PGs can also be observed in platelet aggregation, where TXA₂ activation of the TP receptor causes platelet aggregation. Conversely, prostacyclin activates platelet IP receptors which oppose this platelet aggregation. Thus, the balance of PGs synthesized as well as the complement of PG receptors expressed determines the COX effect on platelet function.

In addition to the GPCR mediated effects, there is evidence that prostaglandin metabolites are capable of activating some nuclear transcription factors. This action is exemplified by the cyclopentenone prostaglandins of the J-series, for example, 15-deoxy-Δ¹², 14-PGJ₂ (15d-PGJ₂) which is derived from PGD₂. 15d-PGJ₂ activates a nuclear hormone receptor designated peroxisome proliferator activator receptor gamma (►PPAR-γ). There is also evidence that prostacyclin (PGI₂) also activates another member of this family designated PPAR-δ. It is unclear whether the PGs represent true endogenous ligands of these receptors, as their affinity is generally in the micromolar range, two to three orders of magnitude lower affinity than the nanomolar affinities observed for PGs at the GPCRs, yet nonetheless concentrations that could be achieved in the intracellular environment.

Prostanoids. Table 1 Pharmacological properties of prostanoid receptors

Class	Subtype	Agonists	Antagonists	Signaling	
TXA ₂	TP	U46619, I-BOP, STA ₂	SQ29548, S-145, ramatroban	G _q , G _s (α), G _i (β), G _h (α), G ₁₂	↑ IP ₃ /DAG/Ca ²⁺ , ↑ cAMP, ↓ cAMP
PGD ₂	DP	BW245C, L-644,698, ZK110841	BWA868C*, S-5751	G _s	↑ cAMP, ↑ Ca ²⁺
	CRTH2	DK-PGD ₂ , indomethacin, 15-R-methyl-PGD ₂ , 15d-PGJ ₂	ramatroban	G _i	↓ cAMP, ↑ Ca ²⁺ , PLC, PI3-K, MAPK
PGE ₂	EP ₁	ONO-KI-004, iloprost, 17-phenyl-trinor PGE ₂ , sulprostone	SC51322, SC51089, ONO-8713	?	↑ Ca ²⁺
	EP ₂	butaprost, 11-deoxy PGE ₁ , AH13205, ONO-AEI-259		G _s	↑ cAMP, EGFR transactivation, β-catenin
	EP ₃	sulprostone, MB28767, misoprostol, SC46275, ONO-AE-249	ONO-AE3-240, L-826266	G _i , G _q , G _s	↓ cAMP, ↑ IP ₃ /DAG, ↑ cAMP
	EP ₄	PGE ₁ -OH, misoprostol, ONO-AEI-329	AH23848B, ONO-AE3-208	G _s	↑ cAMP, PI3-K, ERK1/2, β-catenin
PGI ₂	IP	iloprost, cicaprost, carbacyclin		G _s , G _q , G _i	↑ cAMP, ↑ IP ₃ /DAG, ↓ cAMP
PGF _{2α}	FP	fluprostenol, latanoprost		G _q	↑ IP ₃ /DAG, Rho, EGFR transactivation, β-catenin

*Partial agonist



Prostanoids. Figure 2 EP₃ receptor sequence of three mouse EP₃ receptor splice variants differing only in their intracellular carboxyl termini. The predicted amino acid sequences of each splice variant are represented by the one letter amino acid code. The common region is comprised of two exons, which are spliced to three possible C-terminal tails. The carboxyl variable tails are designated alpha, beta, and gamma, each encoded by distinct exons.

Prostanoid Catabolism

Prostaglandins are short lived and endogenous PGs circulating at extremely low levels. Once synthesized and released, PGs are rapidly inactivated by one pass through the pulmonary circulation. The principal inactivating step of PGs is the oxidation of the 15-OH group to the corresponding ketone by prostaglandin 15-OH dehydrogenase [4]. Consequently many

synthetic PG analogs have modifications at the 15 carbon to decrease inactivation of these compounds and increase their half-life in vivo.

Drugs

Two distinct classes of drugs are important in modulating PG signaling: those that inhibit PG synthesis and those that act directly on the receptor as either agonists

or antagonists. Drugs that inhibit PG synthesis are a particularly widely utilized class of therapeutic agents designated as ►**non-steroidal anti-inflammatory drugs** (NSAIDs). These drugs act as either competitive inhibitors of COX (e.g., ibuprofen, indomethacin, diclofenac) or as irreversible inactivators of COX enzymes (aspirin). Classical nonselective NSAIDs inhibit the cyclooxygenase activity of both COX-1 and COX-2, thereby suppressing PG synthesis. These drugs are utilized for their antipyretic, anti-inflammatory, and analgesic properties, and as a class, they represent one of the most widely prescribed and economically important groups of drugs. Although generally safe and effective, because of their widespread use, significant numbers of patients develop undesirable side effects from these drugs. The most common serious side effects are gastrointestinal bleeding and renal failure. PGE₂ is a major PG in the gastrointestinal (GI) tract, and NSAID mediated suppression of PGE synthesis is thought to be responsible for NSAID induced gastrointestinal injury. Coadministration of the PGE₂ analog misoprostol with NSAIDs is cytoprotective. Nonetheless, misoprostol itself can have unwanted GI side effects such as cramping and diarrhea. An alternative approach for developing NSAIDs with decreased gastrointestinal side effects has been the identification of isozyme selective inhibitors that selectively target the COX-2 enzyme, while leaving the COX-1 isozyme functional. COX-2 expression is inducible, and its expression level is elevated in response to many inflammatory stimuli. COX-2 selective inhibitors such as valdecoxib, rofecoxib, and celecoxib have significantly reduced the unwanted side effects of NSAIDs such as gastrointestinal bleeding, while retaining their antipyretic, analgesic, and anti-inflammatory properties [5]. Moreover, these drugs can be administered at doses sufficient to allow essentially complete blockade of COX-2 without the acute side effects of dual COX-1 and COX-2 blockade. Clinical studies have identified a unique profile of unwanted side effects of selective inhibition of COX-2, resulting in the withdrawal of many COX-2 selective inhibitors from the market. Selective COX-2 inhibition has been associated with an increase in cardiovascular events including stroke and myocardial infarction. The observation that prothrombotic platelet derived TXA₂ is primarily synthesized by COX-1, whereas antithrombotic prostacyclin is, at least in part, a COX-2 product may underlie this profile of side effects.

Because of the complexities resulting from the use of these relatively broadly acting COX inhibitors, the use of selective PG synthase inhibitors or receptor agonists and antagonists could provide therapeutic alternatives devoid of these side effects and therefore with therapeutic value. At this point, there are no potent and selective PG synthase inhibitors or receptor

antagonists in routine clinical use [1]. Development of selective agonists for PG receptors has been limited. Use of PG receptor agonists is complicated because these ►**autacoids** normally act locally and systemic administration of PG agonists may have profound adverse side effects. Nonetheless, several PG agonists are in clinical usage, and they are particularly effective when they can be delivered directly to the site of action.

For example, PGF_{2α} agonists such as latanoprost have been developed as eyedrops to reduce intraocular pressure for the treatment of glaucoma. Topical instillation of these agonists is effective in lowering intraocular pressure and may be used as a first-line therapy for the treatment of glaucoma.

PGE₂ was initially characterized by its actions on female reproductive tissue, and this has been an area of considerable pharmaceutical development activity. PGE₂ (dinoprostone) and PGE₁ (alprostadil) have been used as abortafacients in early pregnancy, and they facilitate labor at term by promoting ripening and dilatation of the cervix. PGE₁ may be used for the treatment of impotence by direct intracavernous injection. As noted above, the PGE₁ analog, misoprostol, has been utilized for the treatment of NSAID induced GI bleeding and presumably acts by replacing the loss of the endogenous PGE₂.

Prostacyclin (epoprostanol) is one of the few drugs effective for the treatment of Primary Pulmonary Hypertension (PPH) a rare but frequently fatal illness of young adults. Increased blood pressure in the pulmonary circulation leads to right-heart failure. Continuous infusion of epoprostanol leads to a decrease in blood pressure; however, it is unclear whether this is due to direct dilator activity of the IP receptor acting on smooth muscle, or a more indirect mechanism.

It is worth noting that in many cases, PG drugs have found greatest use where they may be applied locally and directly rather than systemically. Glaucoma is treated with latanoprost eyedrops, induction of labor with dinoprostone vaginal suppositories, GI ulcers with oral misoprostol. In contrast, in treatment of PPH with epoprostanol and use of PG analogs as abortafacients, the drugs are given systemically.

Ramatroban is a PG receptor antagonist in use for the treatment of allergic rhinitis in Japan. This compound was initially identified as a TP receptor antagonist, although more recently it has been determined that it is also antagonizes the CRTH2 (DP2) receptor.

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Protamine Sulfate

Protamine sulfate is a mixture of basic polypeptides isolated from salmon sperm that is used to neutralize heparin in vitro or in vivo.

► Anticoagulants

Protease-activated Receptors

Synonyms

PAR-1, PAR-2, PAR-3, PAR-4

► Proteinase-Activated Receptors

Proteases

Proteases (proteinases, peptidases, or proteolytic enzymes) are enzymes that break peptide bonds between amino acids of proteins. The process is called peptide cleavage, a common mechanism of activation or inactivation of enzymes. They use a molecule of water for this, and are thus classified as hydrolases.

► Calpains
 ► Viral Proteases
 ► Antiviral Drugs
 ► Non-viral Peptidases

Proteasome

Multiprotein complex that catalyses ATP-dependent degradation of proteins tagged with ubiquitin.

► Protein Kinase Inhibitors

Protein Degradation

► Ubiquitin/Proteasome

Protein Folding

Proteins fold on a time scale from μ seconds to seconds. Starting from a random coil conformation, proteins can find their stable fold quickly, although the number of possible conformations is astronomically high.

► Bioinformatics
 ► Molecular Modeling

Protein Folding Problem

Proteins fold on a time scale from μ s to s. Starting from a random coil conformation, proteins can find their stable fold quickly although the number of possible conformations is astronomically high. The protein folding problem is to predict the folding and the final structure of a protein solely from its sequence.

The protein structure prediction problem refers to the combinatorial problem to calculate the 3D structure of a protein from its sequence alone. It is one of the biggest challenges in structural bioinformatics.

► Bioinformatics

Protein Kinase

Enzyme that catalyses the transfer of the γ -phosphoryl group of ATP to acceptor hydroxyl groups of serine, threonine and tyrosine residues in the protein.

- Table Appendix: Protein Kinases
- Protein Kinase Inhibitors
- Tyrosine Kinases

Protein Kinase A

Protein kinase A (PKA) is a cyclic AMP-dependent protein kinase, a member of a family of protein kinases that are activated by binding of cAMP to their two regulatory subunits, which results in the release of two active catalytic subunits. Targets of PKA include L-type calcium channels (the relevant subunit and site of phosphorylation is still uncertain), phospholamban (the regulator of the sarcoplasmic calcium ATPase, SERCA) and key enzymes of glucose and lipid metabolism.

- β -Adrenergic System
- Adenylyl Cyclases

Protein Kinase C

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Synonyms

C kinase; PKC

Definition

Protein kinase C (PKC) is an enzyme family whose members are activated by agonists that cause receptor-mediated generation of lipid second messengers. The activated enzymes transduce information from such agonists by phosphorylating relevant downstream substrates.

Basic Characteristics

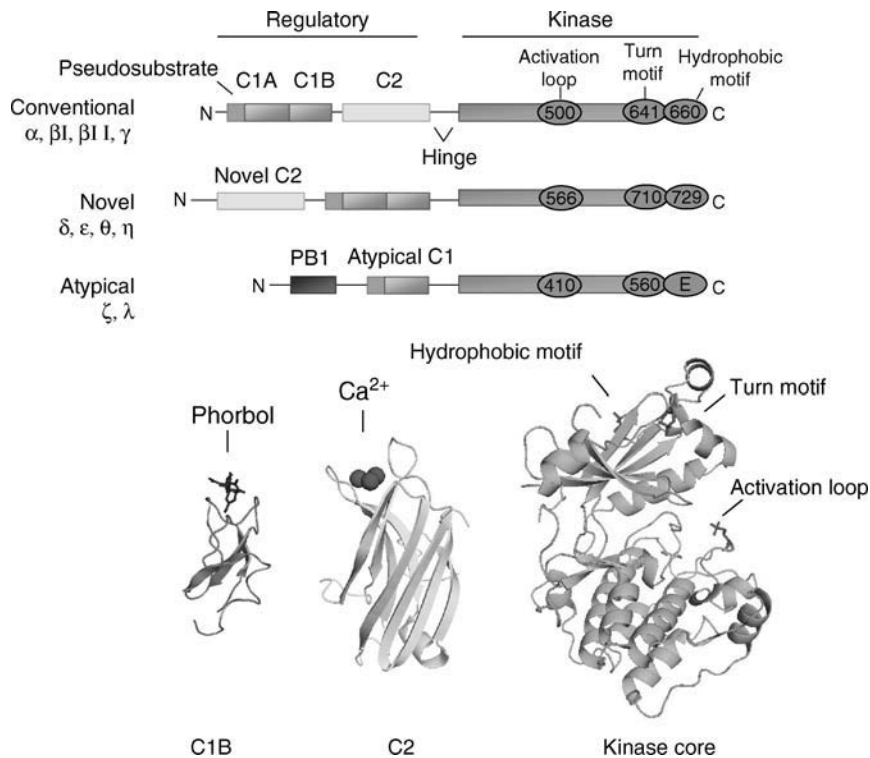
PKC is a family of enzymes whose members play central roles in transducing information from external stimuli to cellular responses. Members of this family of serine/threonine kinases respond to signals that cause lipid hydrolysis. PKC isozymes phosphorylate an abundance of substrates, leading to both short-term cellular responses such as regulation of membrane transport and long-term responses such as memory and learning.

The PKC Family

There are ten mammalian PKC isozymes that fall into three classes: conventional (α , γ , and the two alternative splice variants, β I and β II, which differ only in the C-terminus), novel (δ , ϵ , η , θ), and atypical (ζ , λ) PKC isozymes [3]. As shown in Fig. 1, all isozymes comprise an N-terminal regulatory moiety and a C-terminal kinase core. The regulatory moiety contains two important functional segments: an autoinhibitory pseudosubstrate sequence that allosterically regulates access to the substrate-binding cavity, and one or more membrane-targeting modules. It is the nature of the membrane-targeting modules that defines the classes of PKC isozymes. All PKCs have a version of the C1 domain, the ►diacylglycerol sensor. This domain binds diacylglycerol and the potent functional analogues, phorbol esters, in all isozymes except atypical PKCs. For these isozymes, an impaired ligand-binding pocket does not support the binding of diacylglycerol or phorbol esters and, as a consequence, the hallmark of atypical PKCs is their complete lack of response to phorbol esters. Rather, these isozymes appear to be regulated following activation of PI 3 kinase. They also contain a PB1 (for Phox and Bem1p) protein interaction domain. Conventional and novel PKCs have a C2 domain; this domain binds anionic lipid in a Ca^{2+} -dependent manner for conventional PKCs. However, an impaired Ca^{2+} binding pocket in the novel PKCs makes them unresponsive to Ca^{2+} . Phosphorylation on tyrosine controls protein interactions of this domain in the novel PKC δ .

Regulation

PKC isozymes are regulated by three mechanisms: phosphorylation, second messenger binding, and protein-protein interactions. First, a series of ordered phosphorylations renders newly synthesized PKC catalytically competent. The upstream kinase, the ►phosphatidylinositol kinase-1 (PDK-1), phosphorylates the activation loop of PKC, triggering phosphorylation at two conserved sites in the C-terminus, the turn motif and the hydrophobic motif, the latter occurring via intramolecular autophosphorylation. The positions of these sites on the recently solved structure of the kinase domain of PKC β II are shown in Fig. 1. Second, the mature, fully phosphorylated species of PKC is allosterically activated following engagement of the membrane-targeting



Protein Kinase C. Figure 1 Domain structure of PKC family members showing regulatory modules (pseudosubstrate sequence and C1, C2, and PB1 domains) and the kinase core. Shown below are the structures of the C1 domain of PKC δ with bound phorbol (purple), the C2 domain of PKC β with bound Ca^{2+} (pink spheres), and the recently solved structure of the kinase domain by Grant and coworkers [1] of PKC β II with phosphorylation sites indicated in pink. Figure adapted from Newton (2003).

modules to the membrane. For conventional PKCs, Ca^{2+} pretargets PKC to the membranes by binding the C2 domain and increasing this domain's affinity for anionic lipids. At the membrane, the C1 domain binds DAG, an event that provides the energy to release the autoinhibitory pseudosubstrate sequence from the substrate binding cavity. Novel PKC isoforms respond to DAG alone because their C1 domain has a sufficiently high-affinity binding to DAG such that pretargeting by the C2 domain is not required. Unlike the diacylglycerol-regulated isoforms, the priming phosphorylations of the atypical PKCs appear to be agonist-driven, and phosphorylation by PDK-1 is, so far, the best characterized activation mechanism of these kinases. Third, scaffold proteins position PKC near its activators or substrates, allowing specificity in signaling by distinct isoforms.

Signaling by PKC is terminated by concentrations of its ligands dropping to basal levels (i.e., Ca^{2+} and diacylglycerol) and by dephosphorylation of the three processing sites. Dephosphorylation is controlled, in part, by a recently discovered hydrophobic phosphorylation motif phosphatase. This phosphatase, **PHLPP** (for PH domain Leucine-rich repeat Protein Phosphatase) dephosphorylates conventional and novel PKC isoforms, initiating their downregulation.

Function

PKC isozymes are involved in a wide array of diverse cellular functions [5]. Most isozymes (e.g., PKC β) are involved in proliferative responses, and hyperactivation with phorbol esters most typically results in cell growth and differentiation. However, isozymes can have opposing functions. PKC δ is well-characterized as an apoptotic kinase, whereas the closely related PKC ϵ is antiapoptotic. PKC also plays a key role in learning and memory.

Atypical PKC isozymes have more clearly defined roles. Their best characterized function is in regulating cell polarity by binding two polarity proteins, Par3 and Par6. Consistent with a key role in cell polarity, knockout of PKC λ is embryonic lethal. The atypical PKC isozymes also play a role in glucose homeostasis. These isozymes control insulin-stimulated glucose transport in muscle and adipocytes by promoting the translocation of glucose transporters to the plasma membrane. In liver, atypical PKC isozymes promote insulin-dependent lipid synthesis.

Pathophysiology

The levels of PKC in cells control the amplitude of PKC signaling pathways. Thus, defects in the regulation of PKC that result in altered levels of the kinase impact the

magnitude and duration of downstream signaling. Altered levels of PKC are associated with a number of diseased states, most notably cancer [2]. Here, levels of specific PKC isozymes are frequently upregulated. Best characterized are the roles of PKC β II and PKC ϵ in colon cancer: both isoforms are grossly upregulated in this disease. These isozymes promote colon carcinogenesis; mice lacking these enzymes are resistant to carcinogen-induced preneoplastic lesions whereas overexpression in the colonic epithelium results in enhanced colon carcinogenesis. Because of their roles in glucose homeostasis and insulin signaling, defects in atypical PKC signaling contribute both to obesity and Type II diabetes.

Drugs Activators

Conventional and novel PKC isozymes are potently activated by phorbol esters, heterocyclic compounds found in the milky sap exuded by plants of the Euphorbiaceae family. This sap was used medicinally as a counterirritant and cathartic agent over the millennia; we now know that the active ingredients, phorbol esters, specifically bind to the C1 domain, the diacylglycerol sensor described above. In fact, their ability to recruit PKC to membranes is so effective that phorbol esters cause maximal activation of conventional PKCs, bypassing the requirement for Ca^{2+} . This module is found in a number of other proteins in addition to PKC, so the profound effects of phorbol esters on cells are mediated by other proteins in addition to PKC.

Bryostatins are another class of compounds that bind to the C1 domain and result in acute activation of PKC. However, unlike phorbol esters, these marine natural products have antitumor effects. Bryostatins are currently in phase II clinical trials and show promise as anticancer drugs, particularly when combined with other adjuvant therapy.

Inhibitors

A number of inhibitors directed towards the active site of PKC have been developed [4]. Many of these have therapeutic potential and some are in clinical trials. The drug enzastaurin (LY317615) shows selectivity towards inhibiting PKC β and is currently in clinical trials for cancer. This drug has particular potential as a treatment for colon cancer because of the specific role of PKC β II in this disease (see above). A separate PKC β inhibitor, ruboxistaurin (LY333531) has been developed as a drug to treat the microvasculature complications of diabetes; hyperactivation of both PKC β II and PKC β I contribute to diabetic retinopathy and microvasculature complications.

- ▶ Diacylglycerol
- ▶ Phorbol Esters
- ▶ Protein Kinase C
- ▶ Phospholipases

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Protein Kinase Inhibitors

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Synonyms

Phosphate transferring enzyme inhibitors

Definitions

▶ **Protein kinases** constitute a class of enzymes that transfer a γ -phosphoryl group of ATP donor molecules to acceptor hydroxyl groups of serine, threonine and tyrosine residues in proteins. Many protein kinases are components of signal transduction pathways that regulate cellular processes such as division, differentiation, migration and survival. Their perturbed function often contributes to the development of cancer and other diseases. Therefore, molecules that can inhibit the activity of protein kinases, protein kinase inhibitors (PKIs), can be used both in investigating the function of a specific kinase in a particular signalling pathway, as well as in preventing the aberrant action of protein kinases in pathophysiological conditions [1, 2].

Mechanism of Action

After their synthesis (translation), most proteins go through a maturation process, called post-translational modification that affects their activity. One common post-translational modification of proteins is phosphorylation. Two functional classes of enzymes mediate this reversible process: protein kinases add phosphate groups to hydroxyl groups of serine, threonine and tyrosine in their substrate, while protein phosphatases remove phosphate groups. The phosphate-linking

designed as well. However, such PKI have not yet entered clinical trials.

Inhibitors that Interfere with Docking Sites

The RTK activity phosphorylates tyrosine residues within the intracellular domain of the receptor. These phosphorylated residues function as docking sites for proteins that will convey the signal to downstream signal transduction components. PKI can be developed that bind these phosphorylated docking sites in order to abrogate inappropriate downstream signalling.

Inhibitors Targeting Protein Kinase Transcripts

Another strategy to inhibit the expression of a specific protein kinase exists in preventing translation of its transcripts. One mode to accomplish this relies on the use of ribozymes, which are modified RNA molecules that can cut other RNA molecules. Ribozymes consist of a central catalytic domain with RNA degrading activity, flanked by RNA sequences that are complementary to the target mRNA. In this way, a catalytic domain with no particular specificity can be made to cut up the mRNA encoding a specific protein kinase. Another way to thwart translation is by degradation of target RNA by RNA interference (►[siRNA](#)). Synthetic RNA sequences that are complementary to target protein kinase mRNA will form dsRNA structures with their target. These dsRNA molecules will be recognized and digested by specific RNA degrading enzyme complexes.

Competitors of ATP-Binding Sites

Most of the PKIs currently in clinical trials are small molecules that compete for the ATP-binding site [3, 5]. They prevent the phosphate donor ATP to bind to the protein kinase, and hence the target protein will not become phosphorylated and the perturbed signalling can be terminated.

Competitors of Substrate-Binding Sites

The rationale behind substrate-mimicking molecules lies in their ability to compete with the genuine substrate for binding to the protein kinase. They occupy the binding site for the natural substrate, and therefore terminate the signal transduction event that contributes to the pathogenic state of the cell [3]. Thymidylate synthase is an enzyme that is frequently overexpressed in tumours. The substrate-mimicking compound ThymectacinTM (a phosphoramidate derivative of brivudin) inhibits this enzyme and has successfully entered clinical evaluation against colon cancer. Although not a PKI, this example illustrates the therapeutic potential of substrate-mimicking compounds for PKI. Indeed, substrate-binding site compounds have been synthesized that efficiently ablate protein kinase activity in cell cultures, but none of them have been submitted to clinical trials so far.

Bisubstrate Competitors

Bisubstrate analogue inhibitors form a special group of PKIs that mimic both the phosphate donor (ATP) and the acceptor components (serine-, threonine-, and tyrosine-containing peptides). They can provide enhanced specificity for protein kinases as they block both the ATP- and the substrate-binding site of their target. Bisubstrate analogues that effectively inhibit the insulin receptor tyrosine kinase activity in vitro have been designed, and may thus comprise a new class of therapeutically useful agents.

Inhibitors of Chaperones

Another way to restrain protein kinases is by altering their stability. Heat shock proteins, like e.g. heat shock protein 90 (Hsp90), function as molecular ►[chaperones](#) by binding to various cellular proteins, including protein kinases, thereby regulating the folding, stability and function of their substrates. Thus, chaperone-based inhibitors may prevent the associated chaperone to maintain the activated conformation state of the protein kinase and help to quench the oncogenic activity of this kinase in tumour cells. The inhibition of Hsp90 results in the proteasomal degradation (►[proteasome](#)) of the client protein and in cell death of the targeted cancer cells.

Molecules that Selectively Bind a Specific Conformation of the Protein Kinase

An alternative strategy to inhibit a protein kinase relies on different conformations that active and inactive protein kinase can acquire. Antagonists for a protein kinase can be selected that exclusively bind to the inactive form of the kinase, so as to sequester the molecule in a state that cannot participate in signal transduction.

Clinical Use

Inhibitors that Block the Action of RTK

Antibodies that bind the ligand have been successfully used as inhibitors of RTK [3]. Bevacizumab (Avastin) is a recombinant humanized monoclonal antibody that binds the vascular endothelial growth factor (VEGF), and thereby prevents activation of the VEGF receptor and the assembly of new blood vessels (►[angiogenesis](#)), which ultimately leads to tumour growth regression. Bevacizumab seems beneficial in several clinical trials as an antiangiogenic strategy in cancer patients and has been approved by the Food and Drug Administration as first-line treatment for metastatic colorectal cancer (Table 1). Bevacizumab has low toxicity, but patients possessed an increased risk of encountering thromboembolic events. Ranibizumab (Lucentis, another anti-VEGF monoclonal antibody) is used to treat macular degeneration, a medical condition leading to blindness [3–5]. HerceptinTM (Trastuzumab) is a monoclonal antibody directed against the epidermal growth factor receptor 2 (EGFR2,

Protein Kinase Inhibitors. Table 1 Examples of PKI currently investigated in clinical trials or administered in the clinic

Drug	Type	Target	Disease	Clinical status
Bevacizumab (Avastin)	Monoclonal antibody against ligand	VEGFR	Cancer	Clinic
Herceptin, (Trastuzumab)	Monoclonal antibody against receptor	EGFR2; ErbB-2/neu/HER-2	Cancer	Clinic
ISIS-5132	Antisense RNA	c-Raf	Cancer	Phase II
Angiozyme (RPI4610)	Ribozyme	Flt-1	Cancer	Phase II
Gleevec (Imatinib, STI571)	ATP-analogue	BCR-ABL	Cancer	Clinic
LBH589, 17-AAG	Chaperone inhibitor	Hsp90/BCR-ABL	Cancer	Phase I
KC706	Inhibitor enzyme conformation	MAP kinase p38 α	Inflammatory diseases	Phase II

HER-2, Neu, ErbB-2), which is overexpressed in ~25% of invasive human breast cancer and is associated with an aggressive tumour phenotype and reduced survival rate. Herceptin is now administered to treat metastatic breast cancer and tested in [clinical trials](#) with osteosarcoma and endometrium cancer patients. Most patients do not experience side effects, although some cases of temporary dizziness, fever or chill, headache, skin rash, nausea and shortness of breath have been reported. Despite its initial beneficial response, most patients develop resistance within 1 year. Combined therapy of Herceptin and conventional chemotherapy resulted in a synergistic effect on tumour regression and improved survival rates in patients [1, 3, 5]. Pertuzumab is a monoclonal antibody that blocks dimerization of HER-2 with other EGFR receptors and has entered phase III clinical trials with breast cancer and other solid tumour patients. MDX-447, a bispecific antibody that binds EGFR and immunologic effector cells, is analysed in phase II studies on patients with squamous cell carcinoma of the head and neck. Currently, a phase III trial with MDX-210 (inhibits EGFR2) in combination with monocyte-derived activated killer cell technology against ovarian cancer is under way [3]. To increase the therapeutic potentials of antibodies against RTK, toxic proteins are fused to the antibodies. For example, anti-HER-2 antibodies linked to the fungal toxin maytansine DM-1 are being tested in preclinical studies. Peptides that occupy the receptor are also a strategy to prevent the natural ligand from binding to its receptor. The platelet-derived growth factor (PDGF) receptor is overexpressed in many carcinomas and some cancer patients have elevated serum levels of PDGF compared with healthy individuals. GFB-111 or its second-generation derivative GFB-204 bind the PDGF receptor and block PDGF-induced receptor autophosphorylation and downstream signalling. The GFB compounds

possessed antiangiogenic and anticancer activity against human tumour [xenografts](#) in mice and showed no signs of gross toxicity. However, these compounds still await to enter clinical trials. DAB₃₈₉EGF is a fusion of a specific peptide sequence of EGF and diphtheria toxin. This fusion molecule efficiently binds the EGFR that is overexpressed on tumour cells. DAB₃₈₉EGF was cytotoxic for tumour cell cultures and caused tumour regression in animal models, but no clinical trials have been reported.

Blocking the Phosphotyrosine Docking Sites in RTK

Preventing docking of signalling molecules to the phosphotyrosine motifs in the intracellular domain of the activated RTK may form another mode to interrupt aberrant signalling. CGP78850 can impede the usual SH2-phosphopeptide interactions upon activation of the EGFR, thereby blocking signalling downstream of this receptor. However, CGP78850 has not yet been tested on patients with inappropriate EGFR activity.

Targeting Protein Kinase Transcripts RNA Interference

The antisense oligonucleotide LERafAON against the serine/threonine kinase c-Raf has been tested in phase I clinical trials. The antisense oligonucleotides ISIS-5132, which also inhibits c-Raf, and ISIS-3521, which inhibits PKC, went through different phase clinical trials with solid tumour patients. Unfortunately, no objective responses occurred with these PKI. GEM-231, an oligonucleotide targeting the RI α subunit of protein kinase A is currently undergoing phase I/II clinical trials alone or in combination with traditional therapy for the treatment of solid cancers [3].

Ribozymes

Angiozyme is a ribozyme that specifically recognizes the mRNA for FLT-1, one of the most important VEGF

receptors involved in angiogenesis. Angiozyme, which has gone through phase I and II clinical trials, showed biological activity in metastatic breast cancer, although it could not be used as monotherapy. Angiozyme is now being investigated in combination therapy for metastatic colorectal cancer. Herzyme is also a ribozyme in phase I clinical trial for the treatment of HER2-overexpressing breast cancer [3].

Competitors of the ATP-Binding Site

Imatinib (STI-571 or Gleevec) was one of the first PKI developed to treat Philadelphia chromosome positive leukaemia, and gastrointestinal stromal tumours (GIST). This small compound occupies the ATP-binding site and prevents access of donor ATP to the kinase and subsequent phosphorylation of the substrate. Although the inhibitor was well tolerated in clinical trials and showed high bioavailability and mild side effects, resistance to the drug quickly arose. This resistance lies in functional inactivation of the compound, loss of BCR-ABL kinase target, mutations in the target protein kinase. Second-generation derivatives that circumvent resistance are being developed and tested [1, 3, 4].

Erlotinib (TarcevaTM) competes with ATP in the HER1/EGFR ATP-binding pocket. It is used in the clinic in locally advanced or metastatic non-small cell lung cancer after failure of at least one chemotherapy regime [1, 3, 5].

Inhibitors of Chaperones

Destabilization of an abnormally functioning protein kinase by interrupting the association of the kinase with its chaperone may be beneficial in pathogenic conditions. Encouraging in vitro results with the histone deacetylase inhibitor LBH589 and an analogue of geldanamycin (17-allyl-amino-demethoxy geldanamycin or 17-AAG) demonstrated that both compounds disrupt the chaperone association of Hsp90 with its targets proteins BCR-ABL and mutant FLT-3, resulting in ►ubiquitination and proteasomal degradation of both proteins. Currently, both 17-AAG and LBH589 are in Phase I and II clinical trials as treatment for a variety of solid tumours [3].

Molecules that Selectively Bind a Specific Conformation of the Protein Kinase

KC706 stabilizes the inactive conformation of the mitogen-activated protein kinase p38 α , a protein kinase involved in inflammatory reactions and cardiovascular functions. KC706 therefore holds the potential to treat conditions such as rheumatoid arthritis, psoriasis, inflammatory bowel disease and cardiovascular disease. This compound is currently being tested in phase II clinical trials with patients suffering from rheumatoid arthritis.

► Table Appendix: Protein Kinases

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Protein Phosphatases

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Definition

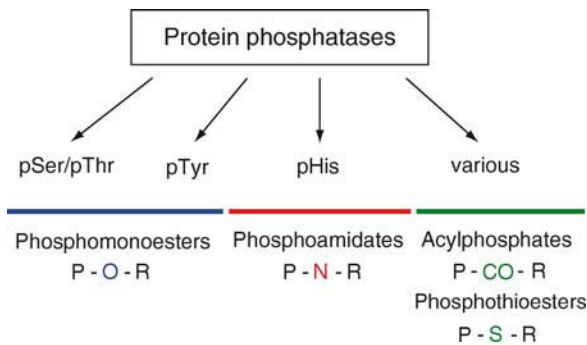
Protein phosphatases are several classes of enzymes that catalyze the hydrolysis of phospho-amino acids within a peptide or protein, thus resulting in dephosphorylation.

Basic Characteristics

Protein phosphatases are obligate counterparts of protein kinases. Originally considered boring enzymes, it is clear by now that phosphatases are playing dynamic regulatory and decisive roles. Protein phosphatases are involved in many fundamental physiological events, including growth, proliferation, survival, differentiation of cells, stress response and the cell cycle, hormone action, ion channel activity, cytoskeleton function, neurotransmission, protein synthesis, gene expression, and metabolic pathways.

Nomenclature

Protein phosphatases are classified according to their activity toward phospho-amino acids they act on (Fig. 1). Nomenclature is independent of regulation simply because stimuli were unknown. Protein phosphatases hydrolyzing *O*-phospho-monoesters are currently subdivided into two major classes: (i) phosphatases acting on phosphoserine (pSer) and phosphothreonine (pThr), and (ii) the second class



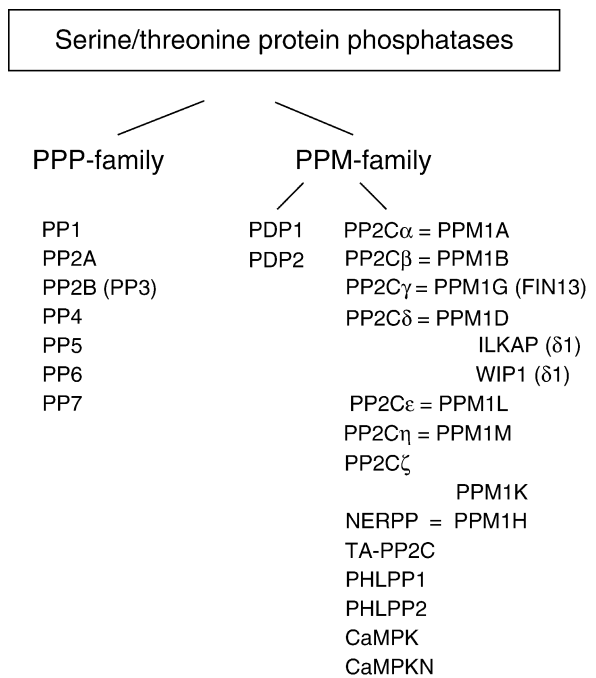
Protein Phosphatases. Figure 1 The diversity of protein phosphatases.

of enzymes acting on phosphotyrosine (pTyr). In addition, there are protein phosphatases hydrolyzing N-phospho-amidates such as phosphohistidine (pHis), phosphatases acting on acyl-phosphates, and those hydrolyzing phospho-thioesters.

Serine/Threonine Protein Phosphatases

Protein phosphatases acting on pSer/pThr were initially defined using biochemical assays as either type 1 (PP1) or type 2 (PP2). Type 1 phosphatases are preferentially inhibited by heat-stable inhibitor proteins while type 2 enzymes are much less affected. The type 2 phosphatases were further subdivided on the basis of divalent cation requirement. PP2A is independent of metal ions whereas PP2B (calcineurin) depends on Ca^{2+} , and PP2C requires Mg^{2+} or Mn^{2+} for activity. Cloning and genetics called for major revision. Nowadays, pSer/pThr protein phosphatases are classified into two groups on the basis of sequence, structure, and catalytic mechanism [1] (Fig. 2): the phospho-protein phosphatase family (PPP), and the family of protein phosphatases that require Mg^{2+} – or Mn^{2+} – ions for activity (PPM).

PPP family members: PP1, PP2A, PP3 (=PP2B, ►calcineurin), PP4, PP5, PP6, and PP7 (Fig. 2). Many of them acquire specificity and are controlled by association with polypeptides that not only localize the enzymes to different cellular compartments but also impart regulation of enzyme activity and substrate recognition. For instance, in higher eukaryotes the catalytic subunit of PP1 interacts with an estimated 200 structurally unrelated proteins to form distinct holoenzymes. Regulatory and targeting subunits typically bind to PP1 via short degenerate sequences, e.g. the RVXF and SILK motifs. Similar complexity is observed with PP2A. Mammals have two PP2A catalytic subunit genes (PPP2C α and PPP2C β – they are not the same as PP2C α and PP2C β of the PPM family, *vide infra*), two structural A subunit genes and four classes of regulatory B subunits with multiple genes in each group. PP2A exists as a heterotrimer. PP4 and PP6 are relatives of PP2A. PP5 has several N-terminal tetratricopeptide



Protein Phosphatases. Figure 2 Families and subtypes of serine/threonine protein phosphatases. PP: protein phosphatases; PPP: phospho protein phosphatases; PPM: protein phosphatases Mg^{2+} -dependent.

(TPR) domains. PP2B (calcineurin) is a heterodimer of a catalytic A-subunit together with a regulatory, Ca^{2+} -binding B-subunit. The A-subunit additionally carries a calmodulin binding site and an autoinhibitory domain. PP7 also contains EF-hand motifs. Both, PP2B and PP7 are stimulated by Ca^{2+} -ions.

PPM family members are characterized by the requirement of Mg^{2+} or Mn^{2+} -ions for activity. The group consists of two pyruvate dehydrogenase phosphatases (PDP1, PDP2) plus 15 protein phosphatase type 2C (PP2C) isozymes (Fig. 2). The 2C enzymes vary in molecular mass, function, and localization. Specific inhibitors are not available. Discovery of the prototypes PP2C α (PPM1A) and PP2C β (PPM1B) of 42 and 45 kD goes back to the 1980s. PP2C isozymes are involved in a variety of processes, e.g., apoptosis, cell cycle, and stress response. Strikingly, at least 23 kinases have been identified as substrates of PP2C enzymes. PP2C members are not evolutionary related to PP1, PP2A, and PP2B, which are all multisubunit enzymes, and its members are not inhibited by okadaic acid.

Protein Tyrosine Phosphatases

Genome sequencing revealed approximately 100 human PTP genes [2], compared with 90 human protein tyrosine kinase genes, suggesting similar levels of complexity among the opponents. The catalytic domain

of all PTPs contains an essential cysteine residue embedded within a conserved signature motif ((I/V)-HCxxGxxR(S/T)) – briefly HC(x)₅R – and catalyzes hydrolysis via a thio-phosphate intermediate.

The classical PTPs can be subdivided into receptor-like PTPs and nonreceptor, cytosolic PTPs. The second category of PTPs are broadly defined as dual specificity phosphatases (DSPs), which dephosphorylate pSer/pThr as well as pTyr. MAP kinase phosphatases (MKPs) (►MAP kinase cascades) and ►PTEN are examples of DSP family members. Remarkably, PTEN also has lipid phosphatase activity that is specific for ►phosphatidylinositol-3,4,5-trisphosphate generated in response to the actions of PI3K. Finally, the class of low molecular mass (LM-) PTPs and that of CDC25 PTPs accomplish the cells repertoire of PTPs (Fig. 3).

Protein Histidine Phosphatases

Histidine phosphatases and aspartate phosphatases are well established in lower organisms, mainly in bacteria and in context with “two-component-systems”. Reversible phosphorylation of histidine residues in vertebrates is in its infancy. The first protein histidine phosphatase (PHP) from mammalian origin was identified just recently. The soluble 14 kD protein does not resemble any of the other phosphatases. ATP-citrate lyase and the β -subunit of ►heterotrimeric GTP-binding proteins are substrates of PHP thus touching both, metabolic pathways and signal transduction [4].

FCPs, SCPs, and HAD Protein Phosphatases

This is an emerging field that has not reached its final position yet. Members of the novel class of FCP, SCP, and HAD phosphatases require Mg^{2+} for catalysis. An aspartate residue within the active site signature motif (DxDx(T/V)) is essential to form an acyl-phosphate intermediate. Many members of the the HAD (haloacid dehalogenase) superfamily have phosphoesterase activity [3]. Some of those protein phosphatases act on

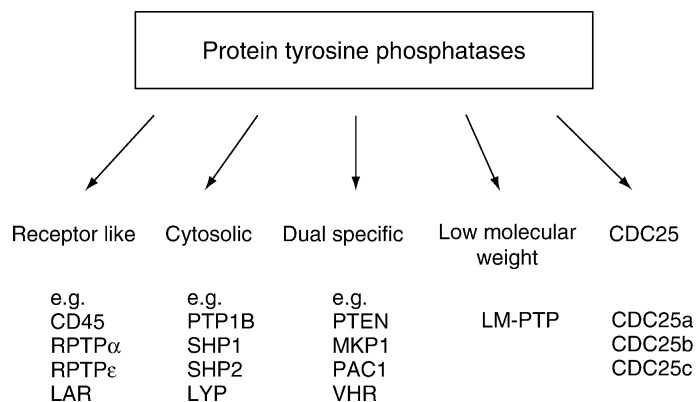
pSer, others on pTyr. Phosphatases dephosphorylating the carboxyl-terminal domain of RNA polymerase II are termed FCPs (TFIIF-associating CTD phosphatase; CTD, carboxyl-terminal domain of the largest subunit of RNA polymerase II). So far all FCPs act on pSer. Small C-terminal domain phosphatases (SCPs) accomplish this novel class of phosphatases. SCPs in addition dephosphorylate the regulatory linker regions of Smad2 and Smad3 to enhance transforming growth factor- β (►TGF β) signaling.

Regulation

For in vitro studies there are a number of compounds available to block protein phosphatase activity. Phosphate buffers inactivate all of these enzymes. Several naturally occurring toxins are potent inhibitors of PPPs, e.g., okadaic acid or microcystin, and are frequently used tools. PPM and PTP family members are not affected by these toxins. Vanadate containing solutions are competitive inhibitors of PTPs, pervanadate is an irreversible inhibitor of PTPs.

In vivo, protein phosphatases are tightly regulated by diverse mechanisms including

- Oxidation (e.g., PTPs; inactivation via oxidation of the active site cysteine)
- Divalent cations (e.g., PP2B, PP7: Ca^{2+} ; PPM-enzymes: Mg^{2+} or Mn^{2+})
- Fatty acids (e.g., PP5; PP2C α and β)
- Methylation (e.g., PP2A; carboxylgroup of the C-terminal Leu-308)
- Dimerization (e.g., some receptor-PTPs such as CD45 and RPTP α)
- Proteolysis (e.g., activation of PP5 by limited proteolysis)
- Phosphorylation (e.g., LM-PTPs, PP1 and inhibition of PP1; the inhibitor protein I1 becomes inhibitory to PP1 only after phosphorylation on Thr-35 by PKA)



Protein Phosphatases. Figure 3 Classification of protein tyrosine phosphatases. See text for details. Common names for selected PTPs are used according to Ref. [2].

- Regulatory subunits (e.g., PP1, PP2A, PP2B; note that inhibitor proteins may, in turn, be further regulated by phosphorylation and dephosphorylation).

Scaffolding Proteins

Numerous protein phosphatases are targeted to their substrates and regulators through the interaction with specific ► **scaffolding proteins**. Some of these anchoring proteins bind both, kinases and phosphatases. This applies to ser/thr protein phosphatases as well as to tyrosine phosphatases.

Drugs and Diseases

The introduction of PP2B (calcineurin) inhibitors revolutionized kidney transplantation. Cyclosporine A and tacrolimus (FK506) are the principal immunosuppressants prescribed for adult and pediatric renal transplantation. Cyclosporine A was in use clinically long before its mechanism of action was elucidated.

Acid- and alkaline phosphatases act on a variety of mono- and multiple phosphate carrying low molecular mass molecules. In addition, they hydrolyze many, but not all, phosphoproteins. They are in use for decades to easily screen for diseases, however, somewhat unspecifically. For instance, acid phosphatase is used as biomarker for prostate cancer, and alkaline phosphatase to monitor bone (de-) mineralization and liver tumors.

In the meantime, aberrant phosphorylation levels of proteins have been linked to many human diseases. The discovery of tyrosine kinases as potential oncogenes had great impact on drug development. Corresponding tyrosine phosphatases can either induce transformation (e.g., oncogenic role of PTP1B in breast cancer) or act as tumor suppressor (e.g., PTEN which is mutated in a large number of cancers). Protein kinase inhibitors are on the market; studying phosphatases as targets has just begun.

Important advances have been made recently from genomic studies. Our understanding of the (patho-) biochemical functions of phosphatases originates from human single-nucleotide polymorphisms (SNPs) and knockout mice. Only few examples will be highlighted here. Knowledge is most advanced within members of the PTP-family. Abnormal PTP genes are also associated with diseases other than cancer. For instance, an SNP of the *PTPN22* gene of lymphoid PTP LYP is associated with diabetes mellitus type I. Variants encoded by the two alleles *1858C* and *1858T* result in a point mutation at position 620 from arginine to tryptophane. This single amino acid exchange is a common risk factor for autoimmune diseases, including type I diabetes and rheumatoid arthritis. Furthermore, PTP1B is an outstanding target for the treatment of diabetes type II and obesity. Antisense-based therapeutics that target PTP1B have normalized blood glucose levels and improved insulin sensitivity, and are now in phase II clinical trial. Among

ser/thr protein phosphatases PP1 and PP2B are involved in neuronal signaling, learning and memory. PP2A accounts for most of the tau phosphatase activity. Abnormal hyperphosphorylation of tau is mainly due to downregulation of PP2A activity in Alzheimer's disease brain. Finally, PP2C seems to be linked to cystic fibrosis, stress response, and atherosclerosis.

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Protein Sorting

► Protein Trafficking and Quality Control

Protein Trafficking and Quality Control

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Synonyms

Protein transport; Protein sorting

Definition

Protein trafficking is the transport of proteins to their correct subcellular compartments or to the extracellular space ("secretory pathway"). Endo- and exocytosis describe vesicle budding and fusion at the plasma membrane and are by most authors not included in the term protein trafficking. Protein quality control comprises all cellular mechanisms, monitoring protein folding and detecting aberrant forms.

Basic Mechanisms

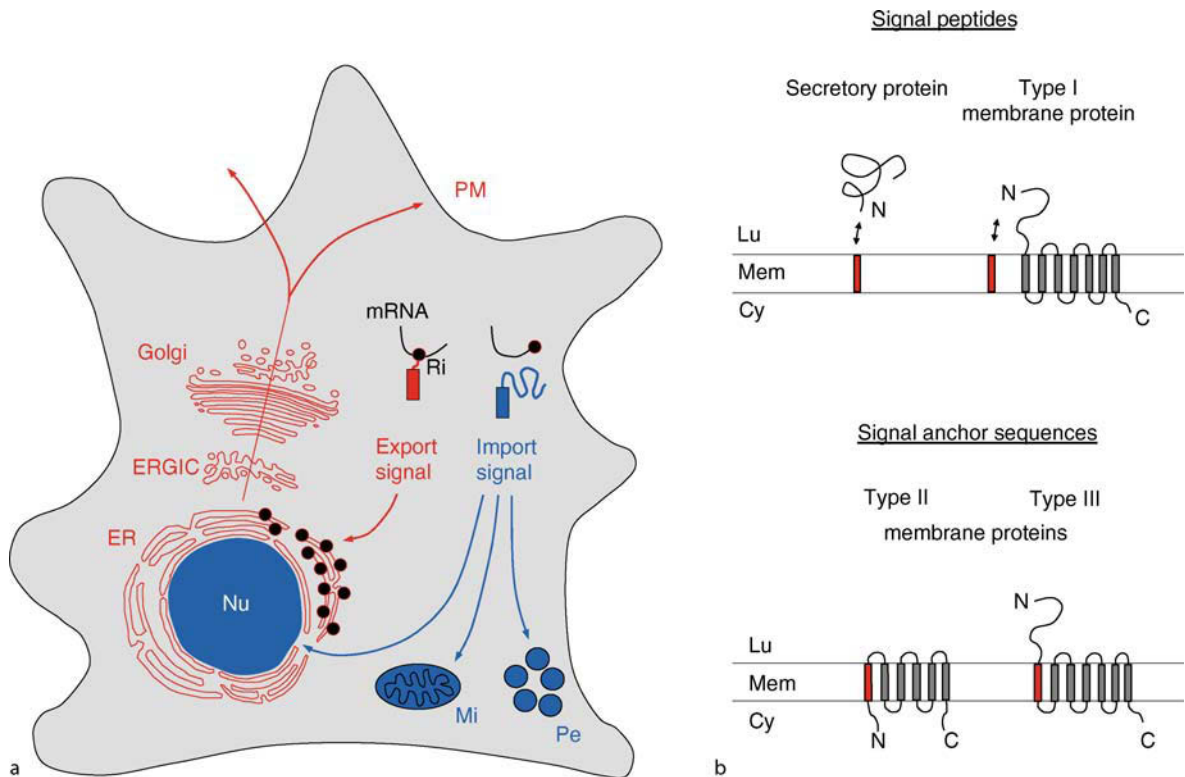
General Transport Routes and Quality Control

The intracellular protein transport and quality control mechanisms ensure that proteins are delivered to their correct subcellular compartment and are thus a prerequisite for both cell architecture and function. Trafficking processes are not only important for proper protein sorting. Together with biosynthesis and degradation mechanisms, they play also an important role in maintaining specific protein concentrations in the various subcellular compartments. Since protein transport is of vital importance, these mechanisms are tightly regulated. Trafficking processes have also drawn the attention of clinicians since many diseases have been shown to be caused by transport deficient proteins.

The pharmacological manipulation of protein folding, trafficking, and quality control is still challenging, but a lot of progress has been made recently.

The general transport routes of proteins are well established (Fig. 1a) [1]. Initially, every protein is synthesized at cytoplasmic ribosomes. Its destination within the cell is then determined by transport signals. If the nascent chain does not contain transport signals, it becomes a cytosolic protein.

Transport signals can be of the import or the export type. Import signals are contained in proteins that are transported into the individual compartments of mitochondria (matrix, inner membrane, intermembrane compartment, outer membrane), peroxisomes (lumen, boundary membrane) and into the interior of



Protein Trafficking and Quality Control. Figure 1 Intracellular protein transport (a) General transport routes of proteins starting with the initial synthesis at cytoplasmic ribosomes (Ri). The export (secretory) pathway to the plasma membrane (PM) via the endoplasmic reticulum (ER), the ER/Golgi intermediate compartment (ERGIC) and the Golgi apparatus (Golgi) is indicated in red. The different import pathways are indicated in blue. Import pathways direct proteins into the individual compartments of mitochondria (Mi) (matrix, inner membrane, intermembrane compartment, outer membrane), peroxisomes (Pe) (lumen, boundary membrane), and into the interior of the nucleus (Nu). Note that transport is posttranslational in the case of all import pathways but cotranslational in the case of the export pathway. (b) Types of signal sequences in the export pathway for translocation across (secretory proteins) or integration into (membrane proteins) the ER membrane. The signal sequences are indicated in red. Lu, ER lumen; Mem, ER membrane; Cy, cytoplasm. Upper panel: secretory proteins contain signal peptides which are removed by signal peptidases in the ER lumen. A subset of the membrane proteins with an extracellular N terminus may also contain signal peptides (type I proteins). Lower panel: the second group of membrane proteins with an extracellular N terminus use signal anchor sequences for ER insertion (type III proteins). Membrane proteins with an intracellular N terminus contain invariably signal anchor sequences (type II proteins).

the nucleus. They mediate transport to the target compartment when protein biosynthesis is complete (posttranslational transport).

Export signals direct proteins to the secretory pathway which runs from the ►endoplasmic reticulum (ER) via the ►ER/Golgi intermediate compartment (ERGIC) and the different compartments of the ►Golgi apparatus to the plasma membrane. The occurrence of export signals leads to the stop of the cytoplasmic protein translation (elongation arrest) by the binding of the ►signal recognition particle (SRP). The resulting nascent chain/ribosome/SRP complex is then targeted to the ►translocon complex at the ER membrane. Here translation restarts and the export signals mediate integration into (membrane proteins) or translocation across the ER membrane (secretory proteins) through the main component of the translocon, the protein-conducting Sec61p channel protein. At the ER membrane, protein integration or translocation takes place while protein synthesis is in progress (cotranslational transport). Secretory proteins contain N-terminal signal peptides as export signals. The signal peptides are cleaved off by the ►signal peptidases of the ER after translocation of the nascent chain. Secretory proteins consequently become part of the ER lumen. In membrane proteins, two different types of export signals were found to mediate ER targeting/insertion (Fig. 1b): (i) proteins with an extracellular N terminus may contain cleavable signal peptides similar to that of secretory proteins (type I proteins); (ii) the vast majority, however, use the first transmembrane domain of the mature protein as a so-called signal anchor sequence (type III proteins). Membrane proteins with an intracellular N tail invariably contain signal anchor sequences (type II proteins). In contrast to secretory proteins, membrane proteins laterally leave the Sec61p protein and thus become part of the ER membrane. Their extracellular domains are translocated into the ER lumen, their intracellular domains remain in the cytoplasm.

In the secretory pathway, the ER is the compartment where protein folding takes place [2]. Proteins called ►chaperone play an important role in protein folding and in the quality control of this process. Chaperones like ►BiP assist in protein folding by their repeated binding to hydrophobic patches of the nascent chain. Moreover, they retain misfolded proteins by their prolonged association, monitor ER stress induced by accumulating aberrant proteins, and induce the unfolded protein response (UPR). The UPR represents a complex adaptation reaction of the cell leading to major changes in transcription and translation of stress-related (increase) and stress-unrelated proteins (decrease). The UPR also induces the ER-associated degradation pathway (ERAD): misfolded proteins are retrotranslocated to the cytosol, ubiquitinated, and finally degraded by the proteasom.

In the case of membrane proteins, the chaperone-based quality control system outlined above seems to be supported or complemented by quality control mechanisms in the ERGIC [3]. Here, subunits of some multimeric proteins (such as ATP-sensitive potassium channels; K_{ATP} -channels) expose short dibasic retrieval signals as long as they are unassembled (consensus sequence = RXR). The RXR signals mediate sorting into retrograde (ERGIC to ER) transport vesicles and consequently reroute the unassembled subunits back to the ER. Upon assembly, the RXR signals are masked and the resulting complex is able to complete the secretory pathway. This system guarantees that only completely assembled multimeric proteins can finalize the secretory pathway. It remains to be determined whether similar quality control mechanisms occur outside the ER and ERGIC, e.g., in the Golgi apparatus.

Once the proteins have passed the quality control system of the early secretory pathway, they are transported in vesicles via the individual compartments of the Golgi apparatus to the plasma membrane. Soluble proteins are transported in the vesicle lumen, membrane proteins are integrated in the vesicle membrane. The transport to the cell surface is the “default” pathway for secretory and membrane proteins. Proteins may also become part of one of the intracellular compartments along the secretory pathway, but only if they contain specific retention signals.

It is thought that soluble proteins enter the transport vesicles of the secretory pathway by bulk flow. In contrast, the recruitment of membrane proteins into the vesicles seems to require sorting signals. Whereas the sorting signals mediating the transport through the intracellular compartments seem to be identical in different cell types, those for the plasma membrane may be variable. In polarized epithelial cells, for example, the cell surface is not homogenous but consists of two different compartments, the apical and the basolateral membranes. Here, additional sorting information is required in the trans-Golgi to deliver proteins correctly. The same holds true for neurons containing dendritic and axonal membranes or endothelial cells, containing luminal and abluminal membranes.

Diseases Caused by Transport-Deficient Proteins

Many diseases are known to be caused by the intracellular retention of mutant proteins or by the impairment of components of the secretory pathway [4]. The disease-causing mechanisms include:

1. Disorders caused by misfolded mutant proteins that fail to pass the quality control system of the ER (e.g., mutations of the cystic fibrosis transmembrane regulator protein (CFTR) causing ►cystic fibrosis). The mutant proteins are retrotranslocated into the cytosol and finally subjected to proteolysis. In some

cases, inefficient proteasomal degradation may lead to the accumulation of proteins in the ER causing severe ER stress and cell damage.

- Disorders caused by the impairment of the Golgi apparatus and further vesicular trafficking (e.g., mutations in the Rab escort protein Rep1 leading to ►[choroideremia](#)).
- Disorders caused by mislocalization of lysosomal proteins (e.g., mutations in the N-acetylglucosamine 1-phosphotransferase leading to ►[inclusion cell disease](#)).
- Disorders affecting the import pathways to mitochondria, peroxisomes, and the nucleus (e.g., mutations in the peroxisomal Pex proteins leading to ►[Zellweger syndrome](#)).

Among the many diseases known to be caused by transport defective proteins, cystic fibrosis is one of the most frequent and best characterized. It is caused by mutations in the gene for the CFTR protein, a cAMP-regulated chloride channel belonging to the ABC transporter family. The CFTR protein is expressed in the apical membrane and in subapical endosomal compartments of secretory epithelial cells, where it regulates the chloride transport over the apical membrane. In about 70% of the patients, the $\Delta F508$ mutation of the CFTR protein is found. The intracellularly retained $\Delta F508$ CFTR protein has some residual function, i.e., the quality control system seems to be overprotective in this case. The $\Delta F508$ CFTR protein is a widely used model for developing drugs which may be useful for the treatment of diseases caused by transport and folding-defective proteins.

Drugs

Most pharmacological strategies for the treatment of protein transport diseases aim to rescue mutant proteins

retained by the quality control system of the ER [5]. The idea is to develop substances favoring correct folding of the mutant proteins, thereby allowing them to pass the quality control system. Although the new pharmacological approaches for the causal treatment of these protein transport diseases are promising, none of the substances made its way out of the experimental or the early clinical trial level as yet. Two groups of substances favoring correct protein folding are examined at the moment: the chemical and the pharmacological chaperones.

Chemical Chaperones

The observation that reduction of temperature sometimes favors correct protein folding led to the idea that this effect may also be achieved by the addition of chemical compounds acting as “chemical chaperones”. Indeed, protein folding can be improved in cells by a wide range of substances acting more or less unspecifically. Osmolytes such as glycerol or trimethylamines may increase solvent density and consequently decrease protein movements and their ability to aggregate. Hydrophobic compounds such as phenylbutyrate (PBA) may prevent protein aggregation by masking exposed hydrophobic domains of misfolded proteins. Aside from their unspecific mode of action, the most important disadvantage of the various chemical chaperones is that they must be used in very high (toxic) concentrations precluding an in vivo application.

Pharmacological Chaperones

The disadvantages of the chemical chaperones led to the idea that correct protein folding may also be stabilized by small compounds binding specifically to the patients target proteins. For example, addition of an agonist or an antagonist to a mutant receptor protein may help to establish the correct conformation of the ligand binding

Protein Trafficking and Quality Control. Table 1 Examples of diseases associated with folding-defective, mutant proteins, and pharmacological chaperones used to correct misfolding in vitro

Disease	Protein affected	Substance
Congenital Long QT syndrome	Human-ether-a-go-go-related protein (HERG)	E-4031, astemizole, cisapride
Cystic fibrosis	Cystic fibrosis transmembrane conductance regulator (CFTR)	Benzo(c)quinolizinium derivatives, VRT-325
Persistent hyperinsulinemic hypoglycemia of infancy (PHHI)	SUR1 subunits of ATP-sensitive potassium channels (K_{ATP})	Sulfonylurea
Hypogonadotropic hypogonadism	Gonadotropin-releasing hormone (GnRH) receptor	GnRH peptidomimetic antagonist
GM2-gangliosidosis	Lysosomal, heterodimeric α -hexosaminidase A (Hex A)	Pyrimethamine
Pompe disease	Acid α -glucosidase (GAA)	Deoxynojirimycin
Retinitis pigmentosa	Opsin	11-cis-7-ring retinal
X-linked nephrogenic diabetes insipidus	Vasopressin V2 receptor (V2R)	SR121463, SR49059

pocket and consequently the correct structure of the full length protein. In nonreceptor proteins, these “pharmacological chaperones” may specifically interact with other protein domains. If such high affinity molecules interact with the extracellular domains of membrane proteins or with secretory proteins, they must, however, be hydrophobic enough to pass not only the plasma but also the ER membrane. At the translocon complex of the ER membrane, transcription, translation, and folding of proteins are closely synchronized processes. Recent data indicate that pharmacological chaperones do not act cotranslationally, but exert their function after translation is complete. It is conceivable that the efficiency of pharmacological chaperones increase with their affinity, the best substances even binding irreversibly to their targets. Thus, studies addressing improved folding and transport of mutant proteins by pharmacological chaperones must always be accompanied by activity and washout experiments.

In the past 5 y, many pharmacological chaperones have been described promoting correct folding of disease-causing, mutant proteins in transfected cells (see [Table 1](#) for examples). It is conceivable that modern high throughput screening methods using activity assays or even automated microscopes will help to indentify many additional substances in the near future.

Neither chemical nor pharmacological chaperones lead to wild-type expression levels of the mutant proteins at the cell surface. Alternative or additional strategies are needed to improve the intracellular transport of the mutant proteins. In the future, drugs may also be developed that influence those components of the quality control system that are involed in the retention of misfolded proteins.

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Protein Transport

►Protein Trafficking and Quality Control

Protein Tyrosine Kinases

►Tyrosine Kinases

Proteinase-activated Receptors

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Synonyms

Protease-activated receptors, PAR-1, PAR-2, PAR-3, PAR-4; Thrombin receptors; Thrombin-related receptors, Trypsin receptor

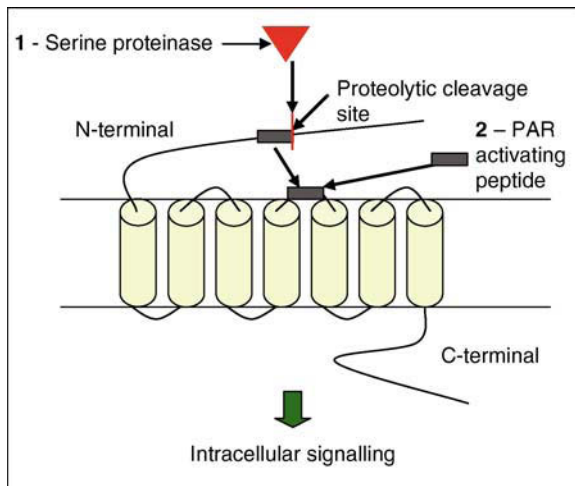
Definition

►Proteinase-activated receptors (PARs) are a unique family of G-protein-coupled receptors (GPCRs) that are activated in response to ►serine proteinases. There are four PAR family members; PAR-1 through to PAR-4. PAR-1 and PAR-3 respond to ►thrombin, PAR-2 responds to ►trypsin, whilst PAR-4 is sensitive to both thrombin- and trypsin-related proteinases.

Basic Characteristics

Mechanism of PAR Activation

PARs are distinguished from other GPCRs by their mechanism of activation. Unlike other GPCRs which are activated reversibly by a freely diffusible ligand, PARs are activated by serine proteinases through irreversible proteolytic cleavage at specific residues upon the N-terminal of the receptor. A new N-terminal ‘tethered’ ligand region is created that interacts intramolecularly with the second extracellular loop (ECL-2) of the receptor to initiate signal transduction via G-protein-dependent pathways ([Fig. 1](#)). PAR-1, PAR-3 and PAR-4 become activated in response to thrombin whilst PAR-2, and to a lesser extent PAR-4, become activated by trypsin-related proteinases. In addition to thrombin and trypsin, other activators of PARs have been reported including coagulation factors, leukocyte and mast cell proteinases, pancreatic and extrapancreatic trypsins and the anchoring protein anticoagulant protein C (APC). Non-mammalian proteinases also display efficacy at PARs, namely those generated from dust mites (Der P3/9), bacteria and fungi ([Table 1](#)). Depending upon the consensus PAR cleavage site and abundance, some proteinases are capable of both activating and deactivating PARs. Cathepsin G can activate PAR-1, however, when cleavage occurs



Proteinase-activated Receptors. Figure 1 Activation of proteinase-activated receptors (PARs) through proteolytic cleavage with serine proteinases (1) and independent of cleavage through PAR-specific activating peptides (2).

downstream of the N-terminal ▶**tethered ligand** region, proteolytic deactivation of the receptor results.

PAR activation can also take place independent of N-terminal cleavage through the direct interaction of synthetic activating peptides (APs) with the extracellular loop. These peptides are derived from the sequence of the freshly cleaved tethered ligand and mimic the intramolecular docking to ECL-2 to induce cell signalling (Table 1). Structure–activity relationship (SAR) studies investigating PAR-activating peptides identified that peptide sequences encompassing the tethered ligand domain as short as 5 or 6 amino acids are adequate for maximal potency. However, in comparison with the nanomolar activity of endogenous proteinases, peptide agonists require micromolar concentrations to elicit a response of comparable magnitude. Furthermore, given the sequence similarity within ECL-2 for all PARs there is considerable potential selectivity problems with peptides previously assumed to be selective for the earlier discovered subtypes. Thus, the development of highly potent agonists and antagonists has been achieved for only PAR-1, whilst for other PAR studies are still ongoing [1].

The Physiological and Pathophysiological Roles of the PAR Family

PARs are coupled to multiple G-proteins and mediate a number of well-defined cellular responses via classical second messenger and kinase pathways. PARs are differentially expressed in cells of the vasculature as well in the brain, lung, gastrointestinal tract, skin as well as other highly vascularised tissues and evidence suggests distinct physiological functions and roles in disease states [2].

PAR-1

Activation of PAR-1 results in intracellular calcium mobilisation and cytoskeletal rearrangement that mediates platelet activation and aggregation. Early studies identified a dual receptor system involving activation of both PAR-1 and PAR-4 in human platelet aggregation. Recent studies have now confirmed that activation of PAR-1/PAR-4 heterodimers act synergistically to activate platelets. In addition to platelets, thrombin also interacts with PAR-1 expressed on other cells of the vasculature such as leukocytes, endothelial cells and smooth muscle cells. In endothelial cells PAR-1 stimulation results in contraction, the activation of von Willebrand factor and up-regulation of cell surface adhesion molecules such as ICAM and VCAM and also tissue factor (TF). Activation of PAR-1 expressed in aorta and coronary endothelial cells mediates relaxation, through the release of nitric oxide (NO) and prostaglandin I_2 (PGI₂), important events in the control of vascular tone. In addition, activation of PAR-1 expressed in vascular endothelial, smooth muscle cells and fibroblasts mediates cellular migration and proliferation through mitogenic pathways, the release of matrix metalloproteinases (MMPs) and subsequent induction of angiogenesis. Thus PAR-1 is intimately involved in the coordination of wound healing responses. Other functions include effects upon the brain, where depending upon the physiological level of thrombin, PAR-1 activation may be both protective against or induce neuronal cell death during trauma.

PAR-2

PAR-2 is strongly expressed in cells of epithelial origin, for example in vascular endothelial cells, airway epithelium, keratinocytes, neutrophils, T cells, synovocytes and chondrocytes. In blood vessels PAR-2 causes relaxation via NO or PGI₂ release, regulates local tissue perfusion, and mediates inflammatory responses associated with wound healing, for example, expression of adhesion molecules. PAR-2 is also strongly expressed in several layers of the skin and has effects upon keratinocyte differentiation and pigmentation via effects upon melanosome incorporation. PAR-2 has multiple effects upon the intestine stimulating prostaglandin release and chloride ion transport in enterocytes and modulating intestinal motility though differential effects upon ciliary and longitudinal muscle. PAR-2 is also expressed in discrete areas of the brain where it activates Ca²⁺ transiently however, in this system the functional role of PAR-2 remains obscure. Whilst a number of studies have implicated PAR-2 as playing a protective role in the airways, coronary vessels and the heart by mediating vessel relaxation, the majority of evidence strongly implicates PAR-2 in inflammation. PAR-2 mediates neurogenic inflammation via release of CGRP and

Proteinase-activated Receptors. Table 1 Proteinase, peptide and non-peptide modulators of PAR activation

Receptor	N-terminal tethered ligand	Proteinase agonists	Peptide agonists ^a	Proteinase/Peptide/Non peptide antagonists
PAR-1	↓S ⁴² FLLRN	Thrombin	SFLLRN (h)	Cathepsin G
		Factor Xa	SFLLRN (r, m)	NP-3
		Plasmin	TFLLRN	HLE ^b
		APC		BMS-200261
		Cathepsin G		RWJ-58259 ^c
		Granzyme A		SCH-79797 ^d
				SCH-205831 ^d
				FR-171113
				P1pal-7 (pepducin)
				P1pal-12 (pepducin)
PAR-2	↓S ³⁵ LIGKV	Trypsin	SLIGKV (h)	Cathepsin G
		Tryptase	SLIGRL (r, m)	Plasmin
		Trypsin IV	2f-LIGKV-NH ₂ ^b	Calpain
		Factor Xa		ENMD-1068 ^b
		TF/VIIa		
		Cathepsin G		
		Acrosin		
		NP-3		
		HLE **Gingipains-		
		RgpA/B		
		Der P3/P9		
PAR-3	↓T ³⁹ FRGAP	Thrombin	None	Cathepsin G
				HLE ^b
PAR-4	↓G ⁴⁸ YPGQV	Thrombin	GYPGKF (m)	YD-3
		Trypsin		
			GYPGQV (h)	tc-YPGKF-NH ₂ ^b
		Plasmin	AYPGKF	P4pal-il (pepducin)
		Cathepsin G		P4pal-10 (pepducin)
		Granzyme A		

↓ denotes cleavage; h, human; m, mouse; r, rat

^aAgonist peptides with amidated (i.e. NH₂) C-terminals are more potent than hydroxylated (OH) peptides

^b2-Furoyl-LIGKV-NH₂; *trans*-cinnamoyl-YPGKF; ENMD-1068—N¹–3-methylbutyryl-N⁴–6-aminohexanoyl-piperazine; HLE, human leukocyte elastase; APC, anticoagulant protein C

^cAnti-thrombotic activity

^dOrally active PAR-1 inhibitors

substance P, whilst studies utilising PAR-2 knockout mice indicate a role in both acute and chronic joint inflammation. PAR-2 also mediates type IV and allergic dermatitis, whilst in the intestine luminal proteinases severely compromise the integrity of the intestinal barrier through PAR-2 activation, implicating a role for PAR-2 in the pathophysiology of the intestinal inflammatory conditions including Crohn's disease and ulcerative colitis. These findings correlate well with a number of cellular events associated with inflammation: release of PAR-2 mediators such as tryptase by mast cells, upregulation of PAR-2 following inflammatory challenge and coupling of PAR-2 to the release of

cytokines and expression of adhesion molecule via pro-inflammatory signalling pathways such as nuclear factor kappa B (NFκB) and the MAP kinases. However, PAR-2-mediated release of prostaglandins from enterocytes, and gastric mucus secretion which may also protect against intestinal inflammation, again exemplifying a potential dual effect.

PAR-3

The PAR-3 subtype of the PAR family is truncated at the C-terminus limiting its potential to signal intracellularly. Furthermore, other than thrombin, there have been no specific activating peptides developed that

result in PAR-3 activation. As a result, exploration into the functional expression of PAR-3 has been limited to scenarios where physiological levels of thrombin exist. Such studies have identified PAR-3 to act as a cofactor for the thrombin activation of PAR-4 in murine platelets, where this dual receptor system results in platelet aggregation in mice. The cooperative interaction between PAR-3 and PAR-4 occurs through thrombin anchoring to the ▶[hirudin](#) domain of PAR-3, where it facilitates in the cleavage of PAR-4.

PAR-4

A number of techniques including Northern blotting, PCR (polymerase chain reaction) and functional studies have shown PAR-4 to be expressed in a variety of cell types including platelets, smooth muscle cells, endothelial cells, leukocytes, cardiomyocytes and neurons. As outlined above, PAR-4 is a low affinity thrombin receptor and functions in synergy with PAR-1 to regulate human platelet activation and interacts with PAR-3 in mouse platelet aggregation. PAR-4 is also expressed in smooth muscle cells where it mediates proliferation and thus contributes to wound healing initiated by thrombin via PAR-1. However in some instances PAR-4 acts in opposition to PAR-1. This has been demonstrated the regulation of contractile motility in the oesophagus, where thrombin activation of PAR-1 mediates muscle contraction, whilst PAR-4 activation results in muscle relaxation, with the dominant effect dependent upon the physiological thrombin concentration. Since the characterisation of PAR-4, many of the original studies investigating possible roles for PAR-1 activation have now been revisited for the possible implication of PAR-4 activation. Such roles include vascular inflammation and cerebrovascular damage. PAR-4 activation results in the release of TNF- α , and activation of MAP kinases and the NF κ B pathway in microglial cells, suggesting a possible role for PAR-4 in inflammation in the brain.

Drugs

The PAR family represents a novel target for drug therapy. Therapeutic approaches with most clinical benefit may rely upon the development of both agonists and antagonists, either of which could be utilised depending upon the disease condition [3].

The most promising prospect of PARs as a therapeutic target remains in vascular biology for the treatment of cardiovascular diseases. Numerous studies have confirmed the potential of PAR inhibition in the treatment of disease states. Several PAR-1 antagonists have been developed ([Table 1](#)), in particular the non-peptide antagonist SCH-205831 currently in phase II/III clinical trials as a candidate for anti-thrombotic therapy. Other strategies under investigation include targeting the PAR-1/PAR-4 heterodimeric complex in human platelets using

PAR1/4-specific antagonists. This dual inhibition strategy has been demonstrated in animal models utilising a combination of PAR-1 antagonist and palmitoylated peptide pepducins which inhibit PAR-4 signalling, suggesting the potential of combination therapy for the management of thrombosis [4]. In addition to thrombosis, antagonist pepducins directed at PAR-1 have also shown potential in targeting PAR-1 mediated breast cancer tumorigenesis and metastasis, inflammation and sepsis. The potential of antagonising PAR-4 in other conditions where PAR-1 is not co-expressed has not been examined, however such antagonism may be an effective anti-inflammatory therapy for the treatment of neurotrauma.

Studies utilising PAR-2 deficient mice have supported the proposed physiological and pathological roles of PAR-2 in a number of disease states. Although research in this area is in progress, PAR-2 antagonists such as novel small molecule antagonist ENMD-1068, may prove to be of therapeutic value in joint inflammation, when used alone or in combination with other therapies. However, ENMD-1068 is of very low-affinity and until high-affinity compounds are developed, other strategies for example, using humanised PAR-2 blocking antibodies may be more successful. PAR-2 antagonists may also prove beneficial in the suppression the proliferative responses that underlie tumour metastasis.

Given the potential protective effects of PARs in some situations the potential of utilising agonists therapeutically has been considered but not as yet exploited clinically. For example, the PAR-2 activating peptide 2f-SLIGKV-NH₂ has been shown to be cytoprotective in intestine and thus prove to be a worthwhile therapeutic target in the treatment of gastritis or gastric ulcers. PAR agonists may also have a limited role in asthma and in ischemic damage [5].

From these studies it is clear that the progression of PAR-based therapy relies upon the future development of new specific agonists and antagonists for PARs 2–4 with much higher potencies than those currently available, with an efficient mode of delivery to target sites. However despite these problems the PARs still represent one of the most attractive therapeutic targets for a number of disease states.

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Proteinases

- ▶ Non-viral Peptidases
- ▶ Viral Proteases

14-3-3 Proteins

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Synonyms

BMH1; BMH2; RAD24; RAD25; Leonardo

Definition

The 14-3-3s are a family of ~30 kDa proteins that exist as homo- and heterodimers within all eukaryotic cells. 14-3-3 proteins bind target proteins containing phosphothreonine or phosphoserine motifs, however exceptions do exist. The docking of 14-3-3s to their target can: (i) cause conformational changes in the target protein, (ii) block sequence-specific or structural features on the target protein, or (iii) function as a scaffold allowing other proteins to dock.

Basic Mechanisms

Recognizing Covalent Modifications

Evolution has provided the cell with a repertoire of 20 amino acids to build proteins. The diversity of amino acid side chain properties is enormous, yet many additional functional groups have been selectively chosen to be covalently attached to side chains and this further increases the unique properties of proteins. These additional groups play a regulatory role allowing the cell to respond to changing cellular conditions and events. Known covalent modifications of proteins now include phosphorylation, methylation, acetylation, ubiquitylation, hydroxylation, uridylation and glycosylation, among many others. Intense study in this field has shown the addition of a phosphate moiety to a protein

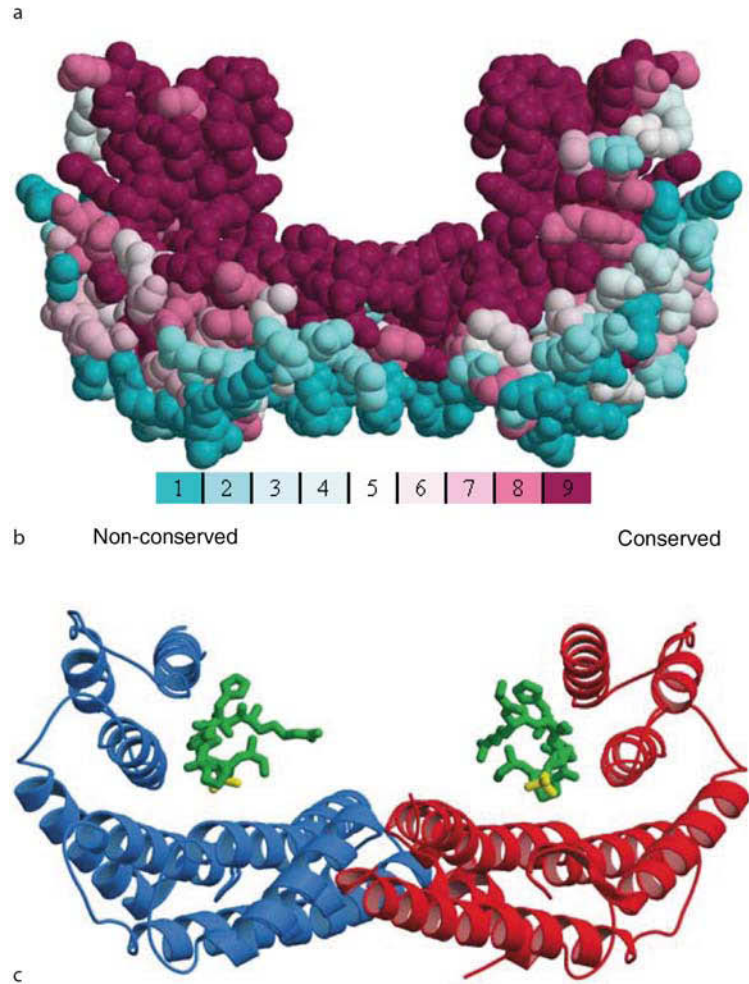
can result in several potential effects including control of enzymatic activity, the shuttling of proteins between cellular compartments and regulation of proteolytic degradation. More recently the study of signal transduction events has provided a new understanding of the regulation of cellular activities by protein: protein interaction and many of the covalent modifications mentioned above perform their function by providing specific docking sites for other proteins. Again, the field to protein phosphorylation was a forerunner in recognizing and characterizing this property of covalent modifications and the 14-3-3 proteins were the first protein shown to specifically bind a phosphoserine or -threonine motif. Thus, the generation of specific docking sites for other proteins is an additional, regulatory function for protein phosphorylation. Other [▶phospho-specific binding modules](#) that can be recognized by amino acid sequence within proteins include leucine rich repeats (LRR), forkhead-associated (FHA), WW, FF, tetratricopeptide repeat (TPR), MH2, WD40, polobox and BRCA1 carboxyl-terminal (BRCT) domains for serine and threonine phosphorylation and Src homology two (SH2) and protein tyrosine binding (PTB) domains for phosphotyrosine. It is likely that additional phospho-specific binding modules exist.

The 14-3-3 proteins were initially catalogued as abundant brain proteins and designated 14-3-3 based on their chromatography column elution fraction and position after starch gel electrophoresis [1]. Defining the role for these proteins began many years later when they were identified initially as activators of tryptophan and tyrosine hydroxylases, and then as regulators of protein kinase C and the protein kinase Raf-1, and as a direct binding protein for the polyoma virus middle T-antigen. This was followed by the key observation that binding of 14-3-3 to its target protein was dependent on the phosphorylation of the target protein. Since this discovery, 14-3-3s have continuously surfaced as regulators of a multitude of phosphorylation dependent cellular events [1–4].

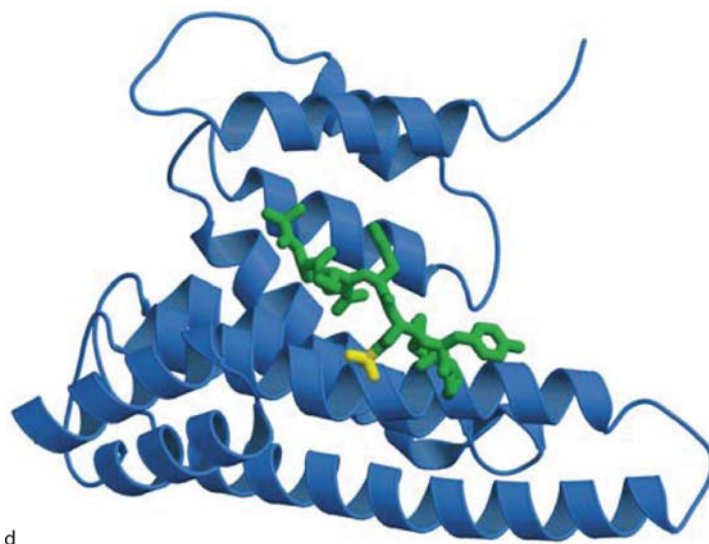
14-3-3 Sequence Conservation, Structure and Function

Cloning and genome sequencing has shown there to be multiple 14-3-3 genes in all eukaryotic organisms with 12 in *Arabidopsis*, 7 in mammals and 2 in *Saccharomyces cerevisiae*, *C. elegans* and *D. melanogaster*. No 14-3-3 or 14-3-3-like genes have been noted any archaeal or bacterial genomes. The *S. cerevisiae* 14-3-3 isoforms are encoded by *BMH1* and *BMH2* and deletion of either does not cause growth defects, but the double deletion of both is lethal. The 14-3-3s are highly conserved across species [Fig. 1\(a\)](#) with divergence occurring on the N- and C-termini. The 14-3-3s have acidic pI values, are small in size (~28–32 kDa) and form homo- and heterodimers. Crystal structures have been elucidated for the unliganded form, and in complex with a high affinity phosphopeptide

sc BMH1	20	40	60	62
at GP14k	68			
dm 14-3-3e	62			
ce 14-3-31	63			
hs 14-3-3Z	61			
sc BMH1	80	100	120	130
at GP14k	135			
dm 14-3-3e	129			
ce 14-3-31	128			
hs 14-3-3Z	126			
sc BMH1	140	160	180	198
at GP14k	203			
dm 14-3-3e	197			
ce 14-3-31	195			
hs 14-3-3Z	194			
sc BMH1	220	240	260	266
at GP14k	248			
dm 14-3-3e	262			
ce 14-3-31	248			
hs 14-3-3Z	245			



14-3-3 Proteins. Figure 1 (Continued)



14-3-3 Proteins. Figure 1 Conservation of peptide binding by 14-3-3 protein. (a) Sequence alignment of evolutionarily diverse 14-3-3 proteins. Residues that are 100% identical are shaded in black, and residues that are >50% identical are shaded in gray. Proteins from *Saccharomyces cerevisiae* (Bmh1), *Arabidopsis thaliana* (GP14κ), *D. melanogaster* (14-3-3ε), *Caenorhabditis elegans* (14-3-3 isoform 1), and human (14-3-3ζ) are included in the alignment. Residues that directly contact the phosphate group of target proteins are indicated with arrows. (b) Structure of 14-3-3ζ shaded according to residue conservation using 14-3-3 isoforms from humans (7 isoforms), *A. thaliana* (12 isoforms), *S. cerevisiae* (2 isoforms), *D. melanogaster* (2 isoforms), and *C. elegans* (2 isoforms). Structures (b, c and d), based on 14-3-3ζ [PDB 1QJB]. In (c) and (d) the peptide (ARSHpSYPA) binds in an extended conformation to 14-3-3 (shown as a ribbon diagram in different shades of gray for each monomer) within the conserved groove of each monomer. Figure from Bridges and Moorhead, *Sci STKE* 2004 RE10. Kindly provided by permission from *Sci STKE*.

from a known target protein. Dimers form a cup shape with the most highly conserved amino acids lining the inside of the cup Fig. 1(b). It is this conserved inner surface that functions as docking site for the phosphoprotein(s). Each subunit has the ability to bind a phosphopeptide and as shown in Fig. 1(c), phosphopeptides dock in an extended anti-parallel orientation. Residues equivalent to Lys⁴⁹, Arg⁵⁶, Arg¹²⁷ and Tyr¹²⁸ of human 14-3-3ζ are completely conserved in every 14-3-3 known and it is these residues that are responsible for direct interaction with the phosphate moiety of the bound phosphoprotein (Fig. 1c, d). It is interesting to note that crystallization of the nonsense mediated decay regulatory protein, ▶SMG7 (suppressor with morphogenetic effects on genitalia 7), revealed structural resemblance of one its ▶TPR domains to one monomer of a 14-3-3 protein even with very limited (<10%) sequence homology. Additional work showed that this region, like 14-3-3 binds peptides in a phosphorylation dependent manner.

When 14-3-3s were first identified as phosphorylation dependent binding proteins (note that a selection of non-phosphorylated targets are known), target protein phosphorylation sites were mapped and it was immediately apparent that 14-3-3s bound preferentially to specific phosphorylation motifs. The advent of oriented

peptide libraries and their application to 14-3-3 binding specificity confirmed this first motif (RSXpSXP; where R is arginine, X is any amino acid, S is serine, pS is phosphoserine and P is proline) that is now described as mode I binding. The peptide library method also pulled out another high affinity binding motif now designated mode II (RXXXpSXP).

In addition to these initially characterized 14-3-3 binding motifs, other studies identified 14-3-3 binding to the C-termini of proteins and this mode or mode III is now recognized as a significant target for 14-3-3s. This motif was first identified in the plant plasma membrane H⁺-ATPase, where the C-terminal sequence QSYpTV-COOH was found to bind 14-3-3 resulting in relief of inhibition of the enzyme by the C-terminus. Binding and relieve of inhibition is dependent upon phosphorylation of the second to last residue; in this case a phosphothreonine. In a genetic screen of random peptides aimed to identify C-terminal signal motifs that override endoplasmic reticulum (ER) retention of proteins destined for the cell plasma membrane, a group of C-terminal peptides that function through an interaction with 14-3-3 were identified and this is much like the sequence of the H⁺-ATPase where the C-terminal sequence. The genetic screen suggested preference for a

hydrophobic amino acid at the C-terminus and a serine or threonine at -2 from the C-terminus. Further work has defined this binding motif as SWpTX_{Pro}-COOH (where pT is phosphothreonine, X is any amino acid except proline and COOH is the carboxyl-terminus of the protein). Interestingly, a number of plasma membrane receptor proteins carry this motif.

Another novel 14-3-3 binding motif was recently uncovered during studies on histones and should be designated Mode IV [5]. Histones are among the most conserved proteins known with covalent modification of their tails, which includes lysine acetylation and methylation, arginine methylation and serine phosphorylation, being responsible for altering access to DNA by charge neutralization and for generating high affinity binding sites for other proteins. Acetylated lysine and methylated lysine residues are known to dock bromo and chromodomains, respectively. Histone H3 is phosphorylated at Ser-10 and 28, which are both preceded by the sequence for Ala-Arg-Lys (ARK). The precise function of this modification was unknown until the N-terminal 20 amino acids were synthesized as unmodified, acetylated (Lys-9 and 14), and acetylated plus Ser-10 phosphorylated peptides and were used for affinity chromatography with HeLa cell nuclear extracts. The only proteins found to bind specifically to the phosphorylated and acetylated (and not acetylated only) versions were 14-3-3 proteins ϵ , ζ and γ . The crystal structure of 14-3-3 ζ complexed with the acetylated and phosphorylated peptide (AR_{Ac}-KpSTGG_{Ac}K, where _{Ac}K is acetylated lysine and pS is phosphoserine) revealed that the basic residues lining the binding pocket as shown in previous structures bind the phosphate moiety. Mode I and II binding motifs have a proline at +2 that allows the bound peptide or protein to change direction and exit the binding cleft. Here positions +2 and +3 have glycine residues that perform the same function. Interestingly, peptides with a glycine at the +2 position were identified as 14-3-3 binding in the original oriented peptide binding studies. The acetyl group at lysine 9 is folded back and hydrogen bonded to the backbone amide. The exit from the cleft after the two glycines means that acetyl-lysine 14 is not in the binding cleft. Although making no contacts with 14-3-3, acetyl-lysine 14 is thought not to be accessible in any way to proteins that bind acetyl-lysine motifs, through motifs such as bromodomains. This structural study nicely demonstrates how 14-3-3 binding can mask another structural feature altering a property of the protein (see below for 14-3-3 roles).

Modes of Action

Early work on 14-3-3 proteins placed researchers in a peculiar position of trying to define the precise function of 14-3-3s. This came from the large number of, and apparently diverse type of targets proteins. Continued work has brought more and more insights and it is

fairly well accepted that 14-3-3s play potentially three functions when bound to a target. They are: (i) 14-3-3 directed conformational changes, (ii) 14-3-3 blockage of sequence-specific or structural features, and (iii) scaffolding.

14-3-3 directed conformational changes. The 14-3-3 proteins are primarily α -helical and are thus described as rigid in nature. The 14-3-3 structure in the presence of phosphopeptides or the target enzyme **serotonin-N-acetyltransferase** support this notion as no change in 14-3-3 shape is observed in the co-crystals. This has led to the molecular anvil or clamping hypothesis for 14-3-3 function whereby the sturdy 14-3-3, upon binding certain targets, cause them to be re-shaped and thus altering their function. This is best described for the enzyme serotonin-N-acetyltransferase. Serotonin-N-acetyltransferase is normally catalytically inefficient and after phosphorylation and 14-3-3 binding displays increased V_{max} and increased substrate affinity. The binding of 14-3-3 causes a local alteration in enzyme, re-shaping the active site. This concept can also be extended to long distance re-shaping of proteins as well where it is thought that 14-3-3 binding can expose or hide protein features or motifs distant from the site binding. For instance, data now support the idea that 14-3-3 binding to several nuclear histone deacetylases (HDACs) causes exposure of a nuclear export sequence (NES) and shuttling from the nucleus.

14-3-3 blockage of sequence-specific or structural features. The physical association of 14-3-3 with a target can also mask or occlude sequence-specific motif or structural features. Examples include the masking of nuclear localization or export sequences and as introduced above, it has been well characterized that the C-terminus of the plant plasma membrane H⁺-ATPase inhibits the activity of the enzyme. After phosphorylation on a mode III motif, 14-3-3 binding removes the tail of the enzyme and the ATPase is activated. Another example of 14-3-3 function by sequence masking has been noted for several proteins synthesized in the endoplasmic reticulum (ER) and destined for the plasma membrane. Many proteins synthesized in the ER are retained there by a diarginine motif that is "recognized" by the coat protein complex I (COPI) retention machinery. Using an affinity-binding method, 14-3-3s were identified as interacting proteins for the dibasic motif of the C-terminus of the potassium channel α subunit, Kir6.2. 14-3-3 Proteins do not recognize the monomeric form of the Kir6.2 tail, but do bind the multimeric Kir6.2 complex, and compete for binding to the dibasic region with the COPI machinery. This suggests that bound 14-3-3 masks the COPI interaction site and thus causes release of Kir6.2 from the ER.

Scaffolding. Because 14-3-3s can bind potentially bind more than one phosphoprotein at once due the presence of two phosphopeptide-binding sites in a

dimer, it has been postulated that they play this role in vivo. Direct observation of this phenomenon is difficult to show, but data from several groups support this idea.

Binding Specificity Among 14-3-3 Isoforms

It is not clear why some organisms have two 14-3-3 isoforms while others have up to 12. Binding 14-3-3 inhibits the plant enzyme nitrate reductase and there appears to be no selectivity between plant 14-3-3 isoforms; in fact yeast and human isoforms appear to work equally as well in vitro. The best example where selectivity has been demonstrated is human ▶14-3-3 σ . 14-3-3 σ Preferential homodimerizes with itself and crystallization revealed a structural basis for this isoform's dimerization properties as well as for its specific selectivity for target binding proteins. Here partner specificity is the result of amino acid differences outside of the phosphopeptide-binding cleft.

14-3-3 Interactome

With more than 350 proteins identified in global 14-3-3 interaction studies the most astounding thing to emerge is the vast repertoire of cellular processes that 14-3-3 plays a role in [2]. Several early studies purified 14-3-3 as an effector molecule that regulated some assayable property of a protein, or 14-3-3 was simply found as a co-purifying protein with some specific target. One of the best-characterized examples is the purification of 14-3-3 as inhibitor of plant nitrate reductase. In the first global study to find new 14-3-3 interactors yeast 14-3-3s (BMH1 and 2) were coupled to a matrix and a plant extract passed over this affinity column to allow proteins to bind. These were affinity eluted with mode I phosphopeptide and released proteins identified. This work found mostly higher abundance metabolic enzymes as the primary targets. More recent studies have employed a similar approach or used specific human 14-3-3-isoforms. Identified targets include proteins involved many metabolic pathways, protein trafficking, signal transduction and transcriptional regulation. Undoubtedly, many more 14-3-3 targets exist and the roles they play in regulating biological function will be uncovered in years to come.

Pharmacological Intervention

The 14-3-3s are abundant eukaryotic proteins that have emerged as key regulators of a vast number of cellular functions. As the list of 14-3-3 interacting proteins grows links with human disease are becoming clearer. Structural studies have defined the interaction surfaces of 14-3-3s with several targets and have now placed them as future potential drug targets. The disruption of protein: protein interactions are now being recognized as a feasible target for pharmacological intervention with several excellent examples demonstrating the feasibility of this approach.

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Proteolytic Enzymes

- ▶ Non-viral Peptidases
- ▶ Viral Proteases

Proteome

The Proteome is the protein complement expressed by a genome. While the genome is static, the proteome continually changes in response to external and internal events.

- ▶ Bioinformatics

Proteomics

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Synonyms

Proteome analysis; Proteome research

Definition

The proteome has been defined as the entire protein complement expressed by a genome. Thus the field of proteomics involves the extensive study of the dynamic protein products of the genome and includes

the identification, characterization and quantitation of proteins and their interactions.

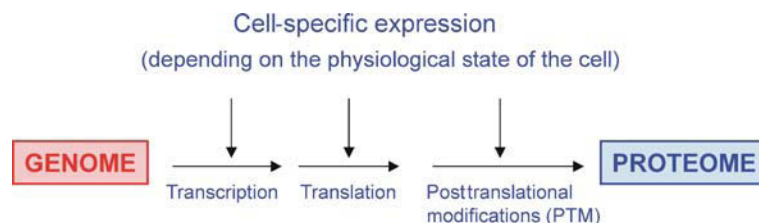
Description

Genome and Proteome: The term proteome was introduced in 1995 [1], and has spread world wide within just a few years. Proteome is the linguistic equivalent of the term genome and describes the whole complement of proteins expressed by a cell at any given time. In contrast to the genome, the proteome is dynamic and very complex (Fig. 1). The expression of a gene has no definitive relationship to the expression or abundance of its protein product. In addition, ►**post-translational modifications** (PTM), such as processing, phosphorylation and glycosylation as well as other modifications cannot be deduced from genomic data. Post-translational protein modifications (PTMs) are important for structure, localization, function, and turnover of proteins, and have been shown to be involved in disease states. Proteomics involves extensive protein analysis and is intimately involved with protein chemistry. In the early work, proteomics was associated with the cataloging of a large number of proteins separated by two-dimensional gel electrophoresis. Now that the human genome and the genomes of several species have been determined, proteomics is expected to contribute to the understanding of gene function. The field of proteomics is currently in a rapid state of development [2]. The main areas of research include: (i) identification of proteins, (ii) determination of details of the primary structure characterization and PTM, (iii) studies of protein interactions and complex formation, and (iv) differential display effects for the comparison of protein expression. Functional proteomics is defined as the use of the methods of proteomics to analyze the molecular networks in cells, for example the identification of specific proteins of these networks following functional stimulation. Large-scale data sets provided by proteomics are needed for the growing field of systems biology research.

The Tools of Proteomics: A variety of methods and techniques including ►**two-dimensional gel electrophoresis** (2DE), capillary liquid chromatography, stable isotope labeling, and mass spectrometry has been developed for qualitative and quantitative protein

analysis. One of the most challenging steps in proteome research is the separation, visualization, and quantification of proteins. Current methodology suffers from the lack of an amplifying method analogous to the polymerase chain reaction. If a given cell at a given time expresses 5,000 genes, approximately 15,000 cellular proteins can be expected as a result of mRNA splicing and PTM. In addition, the broad dynamic range of protein expression (10^8 and higher) and the physico-chemical diversity of proteins makes the visualization of an entire proteome an unresolved problem. Unfortunately, there is no universal separation method, although 2DE is commonly used for the quantitative analysis of the state of expression of large numbers of proteins in a cell. Proteins are separated electrophoretically in the first dimension according to their isoelectric point (isoelectrofocusing, IEF) and in the second dimension according to their mobility in the porous polyacrylamide gel (SDS-PAGE). A standard 2DE of 150 µg of cell lysate allows the resolution of more than 2,000 proteins on the basis of charge and mass. Sample prefractionation techniques prior to 2DE aim to reduce the complexity of protein mixtures found in cells or tissues and may enhance sensitivity for the more interesting low-abundance proteins by removing the house-keeping proteins that are present at higher concentrations. Sensitive staining methods that do not covalently modify proteins are available for the detection of gel-separated proteins. In particular, fluorescent staining methods that offer a broader dynamic range of detection than silver staining, and computer image analysis methods enable quantification of proteins at least over the range of a few orders of magnitude and allow comparative proteome mapping. The weakness of 2DE is that it does not work well with very large, very small, or hydrophobic proteins (especially membrane-bound and cytoskeletal proteins), and those exhibiting extreme isoelectric points. An alternative approach is to use affinity-based protein purification combined with one-dimensional SDS gel electrophoresis, a technique that is able to visualize even extremely hydrophobic as well as acidic and basic proteins.

Nongel separation techniques such as multidimensional capillary chromatography in combination with



Proteomics. Figure 1 Genome versus proteome.

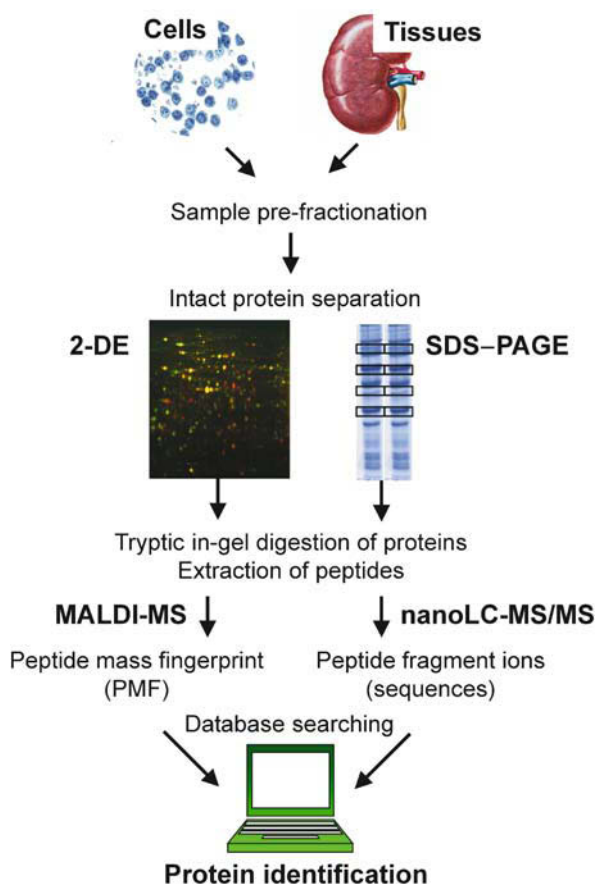
stable isotope labeling methods circumvent the disadvantages of 2DE. Using stable isotope labeling relative quantitation can be achieved by comparing peptide ion intensities which differ only by their isotopic composition. Stable isotope labeling of proteins and peptides can be performed metabolically, chemically, or enzymatically [3]. In the SILAC method (stable-isotope labeling of amino acids in cell culture), two sets of cells are grown independently with one set having ^{13}C -labeled amino acids substituted into the culture media. ICAT (isotope-coded affinity tag) and iTRAQ (isobaric tags for relative and absolute quantitation) are the widely used chemical labeling techniques. The trypsin-catalyzed digestion of proteins in the presence of H_2^{18}O can be used for incorporation of two ^{18}O atoms into the C-terminal carboxyl groups of peptides and does not require chemical derivatization steps. Stable-isotope labeling methods enable quantitative cataloging and comparison of protein expression by mass spectrometry and are therefore useful a wide range of differential proteomics applications. Further advances in proteomics can be expected from the miniaturization of separation techniques (microfluidic chip-based LC) that lead to increased sample concentration, reduced loss of analyte and improved compatibility with mass spectrometry.

The study of proteomes also requires efficient methods for the identification and characterization of separated proteins. Recent advances in mass spectrometric instrumentation and techniques have revolutionized protein analysis; see [4] for review. The development of so-called “soft” ionization methods such as ►electrospray ionization (ESI) and ►matrix-assisted laser desorption/ionization (MALDI) have enabled the generation of gas-phase ions of peptides and proteins without significant fragmentation. Various mass analyzers such as the time-of-flight (TOF), quadrupole, ion trap, linear ion trap, orbitrap, and ion cyclotron resonance (ICR) systems as well as various combinations thereof can be used. MALDI-TOF remains a robust and reliable mass spectrometry (MS) technique for accurate and sensitive mass measurements of peptides as well as high molecular weight proteins. Advanced TOF design and mass analyzer such as ICR and orbitrap have improved the resolution and sensitivity of MS. Triple quadrupole, ion trap, linear ion trap or in particular, hybrid mass analyzers such as quadrupole-TOF, linear ion trap-ICR, and linear ion trap-orbitrap systems have been applied to ►tandem mass spectrometry in which peptide ions are selectively separated in the first step and then fragmented by collision-induced dissociation (CID) and analyzed in the second step in the mass spectrometer. These types of mass spectrometers typically use ESI. MALDI-MS including fragmentation of high-energy ions by TOF-TOF techniques also yield informative fragment ion spectra of peptides. CID spectra obtained by these methods mainly show

sequence-specific N-terminal “b” and C-terminal “y” ions. Such fragmentation by MS gives at least partial information about the peptide sequence and can be used to identify proteins from databases of expressed sequence tags. Very recently new fragmentation techniques have been developed. Implemented on ICR and linear ion trap instruments electron capture dissociation (ECD) and electron transfer dissociation (ETD) will enable a more reliable MS analysis of PTM and intact proteins. In contrast to Edman sequencing, which fails in the analysis of subpicomole quantities or of N-terminally blocked proteins, MS provides accurate mass measurements at a very high level of sensitivity even of protein mixtures. For protein identification the sensitivity limit is in the low femtomole range. Suitable sample volumes are in the range from 500 nL to 1 μL . Although MS is now the method of choice for protein analysis, there is a need for instrumentation that allows greater sensitivity, reliability, and speed. Thus sample preparation methods, the ionization process, mass analyzers, and data processing are fields of rapid development.

Besides sensitive methods for the analysis of proteins, bioinformatics is one of the key components of proteome research. This includes software to monitor and quantify the separation of complex samples, e.g., to analyze 2DE images. Web-based database search engines are available to compare experimentally measured peptide masses or sequence ions of protein digests with theoretical values of peptides derived from protein sequences. Websites for database searching with mass spectrometric data may be found at ►<http://www.expasy.ch/tools>, ►<http://prospector.ucsf.edu/> and ►<http://www.matrixscience.com>.

Identification of proteins, PTM and protein–protein Interactions: Currently, two different methodologies are used for the mass spectrometric analysis of proteins. In the “top-down” approach, the molecular mass as well as backbone fragment ions of intact proteins are measured by high performance MS (ESI-FTICR-MS). Although it has been shown that highly informative fragmentation for proteins with molecular masses greater than 50 kDa could be obtained, the broad applicability of this approach for protein identification and characterization of PTM has yet to be proven. In the “bottom-up” approach, separated proteins or protein mixtures are digested with an enzyme, and the resulting peptides are analyzed by MS and MS/MS methods (Fig. 2). Trypsin is the most commonly used enzyme for digestion since it is inexpensive and the cleavage rules are well known (peptide bonds after lysine and arginine are cleaved selectively). In case of gel-separated proteins, digestion can be performed directly in gel, followed by elution of the peptide mixture. The enzyme normally generates peptides between 600 and 2000 Da, a mass range suitable for sensitive and accurate MS. Once digested, the peptide mixtures can either be



Proteomics. Figure 2 A strategy for mass spectrometry (MS)-based identification of proteins.

directly analyzed by MALDI-MS or can be separated by capillary reversed-phase liquid chromatography coupled to tandem MS instruments such as quadrupole-TOF, TOF-TOF, ion trap, FTICR, or orbitrap. From the mass spectra a set of experimentally obtained peptide masses, the ►peptide mass fingerprint (PMF), can be extracted and used for database searches. Depending on protein molecular weight, the detection of even a small number of peptides (20–30% sequence coverage) provides sufficient data for reliable identification. Peptide mass mapping using MALDI-MS works satisfactorily, if a complete genomic sequence database is available. The method can be automated and makes possible the identification of hundreds of 2DE-separated proteins. In case peptide mass mapping is unsuccessful, tandem MS has to be used. The method utilizes the fragmentation of individual peptides of the protein digest by CID in a collision cell of the mass spectrometer (see above). Protein identification using this approach is particularly useful for proteins from species with incompletely sequenced genomes. MS/MS spectra may yield an amino acid sequence or at least a partial sequence that is specific

for a protein and can be used to search protein sequence databases or nucleotide databases (EST).

More than 200 different naturally occurring PTMs have been described. Most of these modifications are accessible to analysis via MS. Despite numerous reports of MS analysis of phosphorylation and glycosylation, the detection of such modifications in mass fingerprints still presents a serious challenge. Because the modification is frequently labile, MS has to be performed under very mild conditions in terms of sample preparation and ionization. Furthermore, the identification of PTMs requires determination of the specific peptide that contains the modified amino acid. In principle, procedures used for protein identification are also applicable to the determination of PTMs. A mass difference compared to the expected mass after enzymatic degradation of the protein by specific endoproteases (peptide mass mapping) can be used as a sign for the presence of modified peptides, even though this indication is not very specific. Selection and preconcentration of modified sequences by immunoprecipitation or binding to a chelating column are techniques that have been described for phosphopeptides. To determine the site of modification, i.e., the modified amino acid side chain, fragmentation by CID, ECD, or ETD (see above) must be performed.

Because it is well accepted that cellular processes are controlled by multiprotein complexes and such complexes are actual molecular targets of drugs, a further key aim of proteomics is to study protein–protein interactions. The characterization of complexes in which several proteins are associated to perform their biological function can be achieved by purification of the entire protein complex using affinity-based methods, such as glutathione S-transferase (GST)-fusion proteins. The tandem-affinity-purification-tag (TAP-tag) techniques use two high-affinity binding sequences separated by a highly specific cleavage site for a protease to capture and purify a protein complex. After separation by gel electrophoresis proteins are identified by capillary LC-tandem MS. As an example, more than two hundred distinct multiprotein complexes of the yeast proteome [5] as well as several new factors of the human spliceosome were obtained by these techniques and analyzed further.

Pharmacological Relevance

The identification and quantitation of large numbers of proteins by proteomics approaches directly contributes to the validation of diagnostic and prognostic disease biomarkers and to the identification of novel drug targets. Extensive protein identifications has been performed in the framework of numerous proteome projects to study human pathogens (e.g., comparative proteome analysis of *Helicobacter pylori*), to identify disease-associated proteins (e.g., cancer or heart proteomics), and to analyze proteins involved in signal transduction cascades. Although the main goal of

proteomics is the mapping of proteins of cells or tissues, there are further tasks beyond the identification of proteins. PTM of proteins are important for biological processes, particularly in cellular signal transduction. The processing generates functional proteins, lipid modifications determine the location of proteins, and the attachment of phosphate can activate or inactivate reaction cascades. Because of its regulatory importance, protein phosphorylation has received the most attention. Several receptor-mediated signal transduction pathways result in tyrosine phosphorylation of various proteins. A functional proteomics approach that focuses on the isolation, separation, and identification of phosphorylated proteins following stimulation of wild-type and/or modified receptors provides insights into the quality and quantity of signaling proteins. Examples include EGF and PDGF receptor signaling, which involve activation or inactivation by endogenous or exogenous factors and interactions between different signal pathways. Postgenomic proteomics also includes areas such as the determination of protein function, structural analysis, analysis of metabolic pathways, drug mode-of-action and toxicity studies

► Bioinformatics

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Proteosomal Degradation

Proteosomal degradation is the process by which improperly folded proteins or proteins with altered post-translational modifications are removed from a cell before they have a detrimental effect on cellular function. This is performed in small organelles known as proteasomes. Proteins are targeted for destruction in the proteasome by having a number of small ubiquitin molecules added.

- Glucocorticoids
- Ubiquitin/Proteasome

Prothrombin Time

Prothrombin time (PT) is a coagulation assay, which measures the time for plasma to clot upon activation by “thromboplastin” (a mixture of tissue factor and phospholipids).

- Anticoagulants

Protocadherins

- Table appendix: Adhesion Molecules

Protocidal Drugs

- Antiprotozoal Drugs

Proton Pump Inhibitors and Acid Pump Antagonists

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Synonyms

Gastric H,K-ATPase inhibitors; Potassium competitive acid blockers

Definition

The proton pump is the ►gastric H,K-ATPase, which secretes hydronium ions, H_3O^+ , in exchange for K^+ into the secretory canaliculus generating a pH of <1.0 in

lumen of the stomach. Pump-targeted inhibitors of acid secretion can be classified into two groups: covalent inhibitors (proton pump inhibitors, PPIs) and reversible inhibitors (acid pump antagonists, APAs or potassium competitive acid blockers, PCABs). The covalent binding inhibitors are prodrugs, activated by acid catalysis. Their structures are substituted 2-pyridine-methylsulfinyl benzimidazoles. The reversible inhibitors are K^+ -competitive.

Mechanism of Action

The gastric H,K-ATPase consists of a α -subunit of about 1,034 amino acids and a β -subunit having about 290 amino acids. The gastric H,K-ATPase is a membrane protein having ten transmembrane segments in the α -subunit and one transmembrane segment in the β -subunit. The gastric H,K-ATPase transports H_3O^+ ion from cytoplasm to lumen and then reabsorbs K^+ from the lumen due to conformational changes induced by phosphorylation and dephosphorylation of the large α -subunit. In the first step, the E_1 form of the H,K-ATPase binds hydronium ion and MgATP with the ion site facing the cytoplasm. The enzyme is then phosphorylated to form $E_1P \cdot H_3O^+$ and the conformation changes from $E_1P \cdot H_3O^+$ to $E_2P \cdot H_3O^+$ form, where the ion site faces exoplasmically. H_3O^+ is released and K^+ binds on the extracytoplasmic surface of the enzyme, resulting in the $E_2P \cdot K^+$ conformation. This conformation dephosphorylates forming $E_2 \cdot K^+$ with the cation occluded within the membrane domain and then this conformation converts to E_1K^+ that releases K^+ to the cytoplasmic side with binding of MgATP. The H,K-ATPase has a very similar structural motif when

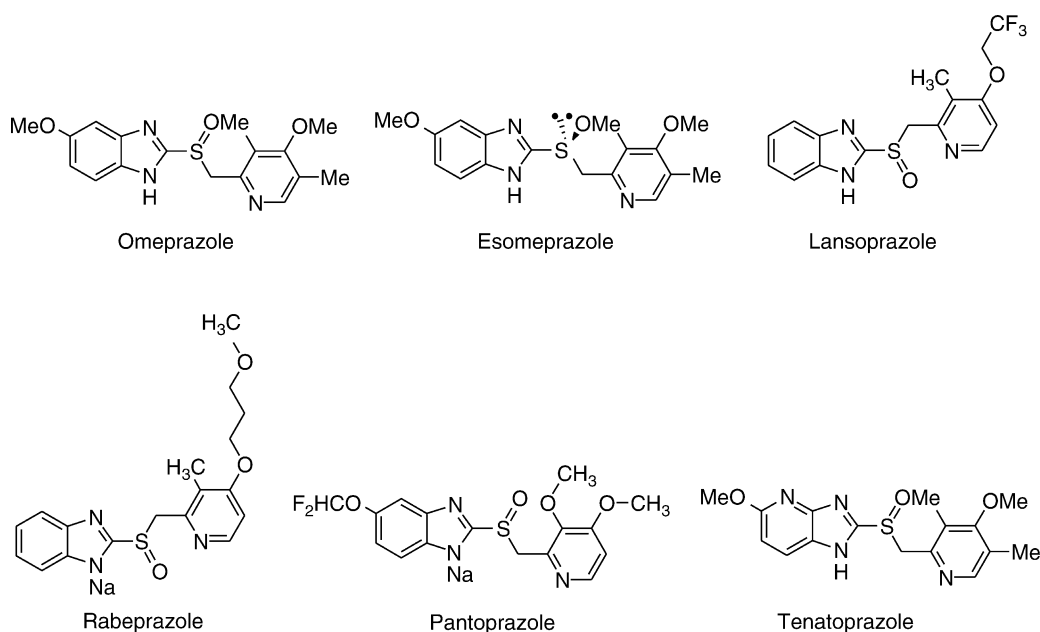
compared with other P_2 -type ATPases such as the Na, K- and Ca-ATPases .

Since a substituted benzimidazole was first reported to inhibit the H,K-ATPase by covalent binding [1], many PPIs have been synthesized and are in clinical use. These all have a similar core structure, 2-pyridylmethylsulfinyl benzimidazole moiety except tenatoprazole. Tenatoprazole has 2-pyridylmethylsulfinyl pyridoimidazole moiety.

It was known that the K^+ -competitive imidazopyridine compound, SCH28080, inhibits acid secretion. Then, many reversible inhibitors were developed. These contain protonatable nitrogens but have a variety of core structures such as imidazopyridines, piperidinopyridines, substituted 4-phenylaminoquinolines, pyrrolo [3,2-c]quinolines, guanidinothiazoles, and 2,4-diaminopyrimidine derivatives. Several reversible inhibitors have been in clinical trials.

Proton Pump Inhibitors

The first compound of this class with inhibitory activity on the enzyme and on acid secretion was the 2-(pyridylmethyl)sulfinylbenzimidazole, timoprazole, and the first pump inhibitor used clinically was omeprazole, 2-[[[3,5-dimethyl-4-methoxypyridin-2-yl]methylsulfinyl]-5-methoxy-1H-benzimidazole. Omeprazole is an acid-activated prodrug. Omeprazole and the other PPIs are accumulated in the acidic space of the parietal cell due to the pK_a of the pyridine nitrogen and these are converted due to protonation of the benzimidazole nitrogen first to a thiol-reactive cationic sulfenic acid and then dehydrated to form the sulfenamide (Fig. 1). These thiophilic cations then bind to lumenally

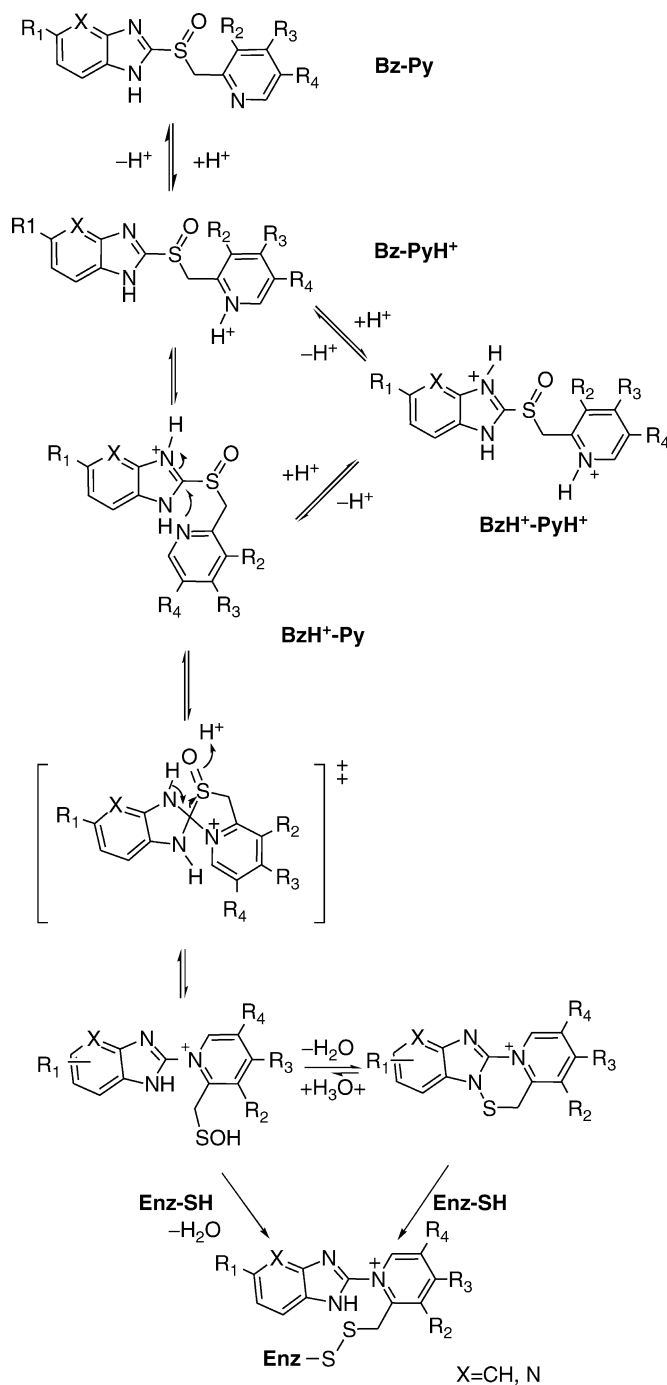


Proton Pump Inhibitors and Acid Pump Antagonists. Figure 1 Irreversible proton pump inhibitors (PPIs).

accessible cysteines of the pump to form disulfides as shown in Fig. 2 [2].

Substituted benzimidazole inhibitors show slightly different effects depending on the inhibitor structure. Omeprazole binds to cysteines in the extracytoplasmic regions of M5/M6 (cys-813) and M7/M8 (cys-892).

Pantoprazole and tenatoprazole bind only to both the cysteines in M5/M6, cysteine 813 and 822, and lansoprazole binds to cysteine 321 in M3/M4 and to cysteine 813 in M5/M6, and cysteine 892 in M7/M8. These data suggest that, of the 28 cysteines in the α -subunit, only the cysteines present in the M5/M6



Proton Pump Inhibitors and Acid Pump Antagonists. Figure 2 Chemical mechanism of irreversible PPIs. PPIs are accumulated in acidic lumen and converted to active sulfenic acid and/or sulfenamide by acid catalysis. These active forms bind to extracytoplasmic cysteines of the gastric H,K-ATPase [3].

domain are important for inhibition of acid secretion by the PPIs [3]. This covalent mechanism extends their duration of action to far beyond their presence in the blood. Also, since active pumps are required and not all pumps can be activated at once, their effect is cumulative, reaching steady state after about 3 days on once a day dosing. The steady state reflects the balance between inhibition of active pumps and restoration by activation of silent pumps after the drug has left the circulation, by de novo synthesis of the pump with a half-life of ~ 54 h and by partial reversal of the enzyme-S-S-inhibitor complex by endogenous glutathione [4].

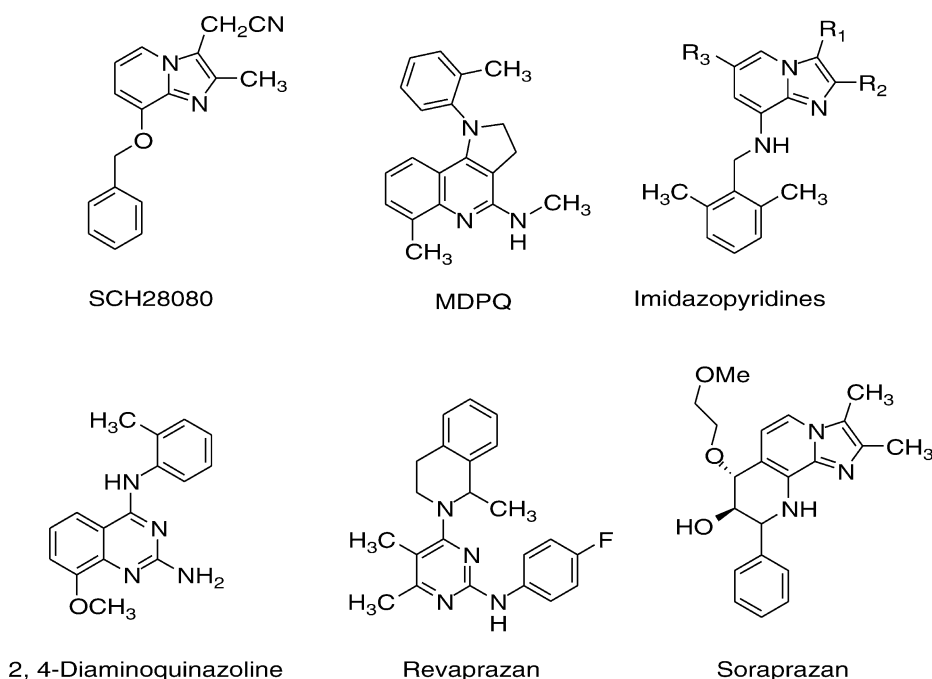
Acid Pump Antagonists or Potassium Competitive Acid Pump Blockers

SCH28080, a substituted imidazo[1,2-a] pyridine, is the best defined among other reversible PPIs. SCH 28080, 3-cyanomethyl-2-methyl-8-(phenylmethoxy) imidazo [1,2-a]pyridine, inhibited the H,K-ATPase competitively with K^+ . SCH 28080 binds to free enzyme extracytoplasmically in the absence of substrate to form $E_2(\text{SCH 28080})$ complexes. SCH 28080 inhibits ATPase activity with high affinity in the absence of K^+ by binding to the E_2P form. SCH 28080 has no effect on spontaneous dephosphorylation but inhibits K^+ -stimulated dephosphorylation, presumably by forming a $E_2P \cdot [I]$ complex. Hence SCH 28080 inhibits K^+ -stimulated ATPase activity by competing with K^+ for binding to E_2P . Steady state phosphorylation is also reduced by SCH 28080, showing that this compound

also binds to the free enzyme. The important difference between this class of inhibitor and the PPIs is that this type does not require acid activation and inhibits the pump by blocking it in mid-cycle (Fig. 3). Hence it is expected that inhibition of acid secretion will be more rapid and more complete. However, the inhibition will last only as long as the drug is present in the blood and therefore will be of shorter duration unless a long dwell time compound is developed or timed release formulation introduced. Soraprazan, one imidazopyridine compound, is under clinical trial and revaprazan, one of 2,4-diaminopyrimidine derivative, is now clinically used in Korea.

Clinical Use

PPIs are used for the therapy of gastric ulcer, duodenal ulcer, ►gastroesophageal reflux disease, ►Zollinger-Ellison syndrome and for eradication of *Helicobacter pylori* in combination with two antibiotics. The primary effect of these PPIs is gastric acid suppression. The degree of acid suppression correlates with healing rates for reflux oesophagitis and peptic ulcer. Omeprazole, lansoprazole, pantoprazole, and rabeprazole show generally equivalent potency of gastric acid suppression. S-omeprazole has become available for clinical use. Omeprazole is a racemate consisting of S- and R-enantiomers. The R-form of omeprazole is sensitive to CYPs 2C19 and 3A4 enzymes, while S-form is less sensitive to these ►CYP enzymes [5]. S-omeprazole has longer plasma half-life compared to omeprazole, providing longer duration of acid suppression when



Proton Pump Inhibitors and Acid Pump Antagonists. Figure 3 Acid pump antagonists (APAs).

given at 40 mg compared to 20 mg of the racemate. Tenatoprazole also has longer plasma half-life, providing longer inhibitory activity.

PPIs are used in triple therapy for eradication of *▶Helicobacter pylori*. Their effect may be due to acid inhibition that results in a greater proportion of the infecting organisms being moved into growth rather than stationary phase. The usual antibiotics used in triple therapy, clarithromycin and amoxicillin (targeting protein or cell wall biosynthesis), require growth phase for efficacy and are not effective against bacteria in stationary phase.

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Proton-sensing GPCRs

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Synonyms

GPR4; Ovarian cancer G protein-coupled receptor 1 (OGR1, GPR68); T cell death-associated gene 8 (TDAG8, GPR65)

Definition

GPR4, OGR1, and TDAG8 are GPCRs regulated by extracellular pH in the physiological range. These receptors are silent at pH 7.8, and fully activated at pH 6.8.

Basic Characteristics

The receptors GPR4, OGR1, and TDAG8 form a subfamily of GPCRs distantly related to purinergic and

angiotensin receptors. They had first been described as receptors for bioactive lipids, however, the publications regarding activation of GPR4 and OGR1 by *▶sphingosylphosphorylcholine* (SPC) and *▶lysophosphatidylcholine* (LPC) have been withdrawn. Activation of TDAG8 by galactosylsphingosine (psychosine) (potential signaling lipid accumulating in krabbe disease. No receptors known.) could not be reproduced in our hands and by other laboratories. Instead, work in our laboratory and by other research groups has established that GPR4, OGR1, and TDAG8 are activated by neutral to slightly acidic extracellular pH [1, 2], suggesting that protons are the activating ligands. Recent developments in this field are summarized in [3]. See Table 1 for overview.

A fourth molecule, the receptor G2A (GPR132), is also related to this group. However, recent data suggest that G2A is a receptor for oxidized free fatty acids. Activation by acidic pH could not be confirmed.

GPR4 and TDAG8 are Gs-coupled GPCRs, they stimulate cAMP formation inside cells. OGR1 is Gq-coupled, leading to activation of phosphoinositide turnover and release of calcium from intracellular stores [1, 2]. The response of these receptors to extracellular pH in recombinant cell systems is shown in Fig. 1. OGR1 appears slightly more sensitive to extracellular protons than GPR4 and TDAG8. In the experimental setup used, halfmaximal activation of receptors was observed at pH 7.45 ± 0.04 for OGR1 (*n* = 14), pH 7.18 ± 0.02 for GPR4 (*n* = 3), and pH 7.15 ± 0.02 for TDAG8 (*n* = 6). As described in [1], the pH values refer to buffers adjusted at room temperature. To obtain pH at 37°C, ca. 0.15 pH units should be subtracted.

To date, we could not identify other GPCRs showing a similar pH-dependent response. Without exception, all receptors studied so far in our hands show a rather stable signal output over the extracellular pH range of 6.5–7.8 following activation by their cognate ligand. However, the existence of other pH-sensing GPCRs is not excluded.

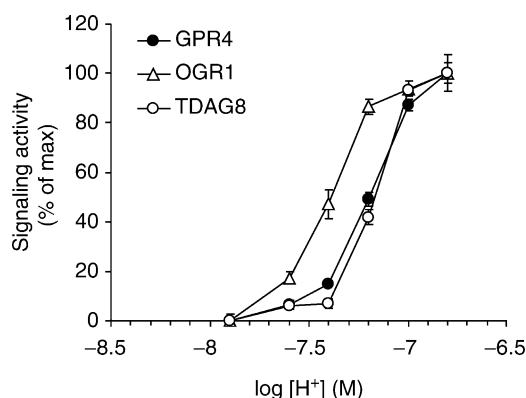
In the light of the earlier studies implying GPR4 and OGR1 as lipid receptors, it was proposed that lipids might act as inhibitory ligands on these receptors. However, as described in detail in [3], our data do not support the notion of a specific modulation of OGR1, GPR4, or TDAG8 by the lipid messengers SPC, LPC, or psychosine.

Structure-Function

OGR1 and GPR4 exhibit a cluster of histidines exposed to the extracellular space, and these amino acids are conserved across mammalian species. Site-directed mutagenesis experiments confirmed the importance of five histidine residues for normal pH sensing of the receptor OGR1 [1]. No detailed studies are yet available for GPR4 and TDAG8. In the latter receptor, three of the histidine residues shown to be relevant in OGR1

Proton-sensing GPCRs. Table 1 pH-sensing receptors

Name	Chromosomal location	Signal transduction	Comment
GPR4	19q13.2–q13.3	cAMP ↑ (G_s)	pH sensor of endothelial cells
OGR1 (GPR68)	14q31	cAMP ↑ (G_s)	pH sensor of osteoblasts, osteoclasts, smooth muscle cells
TDAG8 (GPR65)	14q31–q32.1	IP_3/Ca^{2+} ($G_{q/11}$)	pH sensor of thymocytes



Proton-sensing GPCRs. Figure 1 pH-dependent activation of recombinant receptors expressed in host cells. GPR4 was stably expressed in Hela cells, OGR1 and TDAG8 in CCL39 hamster fibroblasts. For OGR1, formation of inositol phosphates was measured, for GPR4 and TDAG8, cAMP formation was determined. Data were normalized to maximal stimulation (100%). Untransfected host cells show no pH-dependent activation of second messenger formation. Experimental details are as described in [1].

are replaced by basic amino acids. It is possible that these Arg and Lys residues, in the context of other surrounding amino acids in TDAG8, can function in pH sensing.

Potential Physiological Roles of pH-Sensing Receptors

The pH-sensing properties of GPR4, OGR1, and TDAG8 were discovered relatively recent and only limited information is as yet available regarding the physiological function of these receptors. A role in pH homeostasis is likely. Surprisingly little is known today about the molecular mechanisms governing pH homeostasis of higher organisms. Overall control is achieved through regulation of respiration and renal handling of bicarbonate and protons. Bone plays an important role as a reserve for hydroxyl ions, bicarbonate, and phosphate. Cardiovascular function and immune responses are influenced by ▶acidosis.

The three ▶pH-sensing receptors are found in vertebrates – orthologues for OGR1 and GPR4 are detected in zebrafish, chicken, and in all sequenced mammalian species, but not in *Drosophila*, *C. elegans*, or *plasmodium*. TDAG8 is not found in zebrafish, but in the mammalian species analyzed to date. The receptors thus appear to be relevant for physiological processes developed relatively late in evolution.

The first data on genetically modified mice were reported very recently. Animals deficient in GPR4, OGR1, and TDAG8 are viable and fertile, and show no major defects. A short summary of available information is given in the following (see also Table 1).

GPR4 is found in kidney and lung, and recent more detailed analysis showed that it is significantly expressed in endothelial cells. Yang et al. [4] reported that adult $GPR4^{-/-}$ mice appear normal, however, increased perinatal lethality was observed which was associated with vascular abnormalities. Experiments on aortic rings ex vivo showed that microvessel outgrowth was less inhibited by acidic pH in preparations obtained from $GPR4^{-/-}$ mice compared to control. These data indicate that GPR4 functions as a vascular pH sensor.

OGR1 is significantly expressed in lung, kidney, bone, and the nervous system [1 and references cited therein]. We and others demonstrated pH-dependent signaling of the receptor in bone-forming ▶osteoblasts as well as in bone-resorbing ▶osteoclasts [1, 3]. Despite clear evidence for function in these cell types, OGR1-deficient mice show a normally developed skeleton and normal bone mineral density (preliminary data). Further analysis is required to assess the response of the skeleton of $OGR1^{-/-}$ mice to ▶acidosis and hormonal changes. Recently, expression and function of OGR1 were also described in human aortic smooth muscle cells, and the receptor was reported to control acid-induced PGI₂ production. PGI₂ is a vasodilating mediator and may contribute to acidosis-induced vasorelaxation [3].

TDAG8 appears restricted to the immune system. As tissue inflammation is usually followed by local ▶hypoxia and acidosis, the pH-sensing property of TDAG8 appeared of particular interest in this context. Unexpectedly, however, the phenotype of mice

deficient in TDAG8 appears rather mild. The animals develop an apparently normal immune system, and major immune functions are unaffected [5].

Drugs

OGR1, but not GPR4 or TDAG8, is sensitive to inhibition by zinc and copper ions [1]. Agonistic or antagonistic modulators of receptor function are not yet published. As described above, we do not consider lipid messengers as specific modulators of these receptors.

Conclusions

The data available to date identify GPR4, OGR1, and TDAG8, but not G2A, as genuine pH-sensing receptors. Activation by neutral to acidic pH is strong, comparable to activation of other GPCRs by their cognate ligands. The existence of additional specific positive or negative modulators of these receptors can not be excluded, but remains to be demonstrated.

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Proto-oncogene

Normal cellular gene, usually concerned with the regulation of cell proliferation that can be converted to a cancer promoting oncogene by mutation.

► Cancer, Molecular Mechanisms of Therapy

PRRs

Pattern recognition receptors (PRRs) are receptors expressed by cells from the innate immune system

acting as sensors to rapidly detect invading pathogens. PRRs recognize conserved pathogen-associated molecular patterns (PAMPs) and distinguish foreign organisms such as bacteria, viruses, fungi, or parasites, from cells of the host. PRRs are divided into three families. The most studied family is the Toll-like receptors (TLRs). TLRs are membrane proteins anchored in the plasma membrane or at the surface of endosomes. TLRs are characterized by a common ligand-binding domain, which is composed of leucine-rich repeats (LRRs). Their recognition of either extracellular pathogens or PAMPs present in endosomes activates the innate and adaptive immune responses through signaling cascades controlling selective activation of NF- κ B and other inducible transcription factors. The other two families of PRRs, the NOD-like receptors (NLRs) and the RIG-like helicases (RLHs) are soluble receptors present in the cytosol and act as sensors to detect a variety of viral and bacterial products. NOD1 and NOD2 (two NLRs) detect bacterial peptidoglycan while the retinoic acid inducible gene-1 (RIG-1) and the melanoma differentiation associated gene-5 (MDA-5) are RNA helicases that sense viral double-stranded RNA (dsRNA).

► Nuclear Factor Kappa B

► Toll-like Receptors

PSD-95

Synonyms

post-synaptic density 95

Definition

Adaptor protein enriched in the post-synaptic region of neurons. Important for assembly of receptor clusters and signaling complexes.

► Adaptor Proteins

Pseudocholinesterase

► Cholinesterases

Pseudogene

Pseudogenes are nonfunctional relatives of known genes that have lost their ability to encode proteins.

The conversion of a functional into a pseudogene most frequently results from mutations such as frameshift mutations or deletions. Even though pseudogenes might still have some retained functionality such as promoter- or enhancer-like features, they are usually classified as pseudogenes upon their lack of protein-coding ability.

P-Site Ligands

P-site ligands are characteristically adenine nucleosides or adenine nucleoside 3'-phosphates. The term $> \text{P-site} =$ was historically used to describe that $> \text{site} =$ in the adenylyl cyclase system through which a selected class of agents reduced enzyme activity. As originally described, P-site ligands required an intact purine moiety, but tolerated modifications to the ribose group. (This was contrasted with another class of inhibitors that required an intact ribose moiety, but tolerated substitutions to the purine ring. This latter group was later identified and further refined as adenosine and purine receptor agonists.)

P-site ligands inhibit adenylyl cyclases by a noncompetitive, dead-end- (post-transition-state) mechanism (cf. Fig. 6). Typically this is observed when reactions are conducted with Mn^{2+} or Mg^{2+} on forskolin- or hormone-activated adenylyl cyclases. However, under some circumstances, uncompetitive inhibition has been noted. This is typically observed with enzyme that has been stably activated with GTP γ S, with Mg^{2+} as cation. That this is the mechanism of P-site inhibition was most clearly demonstrated with expressed chimeric adenylyl cyclase studied by the reverse reaction. Under these conditions, inhibition by 2'-d-3'-AMP was competitive with cAMP. That is, the P-site is not a site *per se*, but rather an enzyme configuration and these ligands bind to the post-transition-state configuration from which product has left, but before the enzyme cycles to accept new substrate. Consequently, as post-transition-state inhibitors, P-site ligands are remarkably potent and specific inhibitors of adenylyl cyclases and have been used in many studies of tissue and cell function to suppress cAMP formation.

► Adenylyl Cyclases

Psoralens

Psoralens are naturally occurring substances, which are used for photochemotherapy, e.g. for treatment

of psoriasis or vitiligo. The local application of e.g. 8-methoxypsoralen on the skin and subsequent irradiation with UVA-light is called PUVA. Psoralens are absorbed rapidly after oral administration and photosensitivity is maximal about 1–2 hours after ingestion.

Psychedelic Hallucinogen

The term “psychedelic” literally means “mind manifesting” and was applied to hallucinogens like LSD or mescaline to emphasize the intensification of awareness and sensory perception that is associated with these drugs.

► Psychotomimetic Drugs

Psycho Energizers

► Antidepressant Drugs

Psychomotor Stimulant Drugs

► Psychostimulants

Psychostimulants

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Synonyms

Stimulants; Stimulant drugs; Psychomotor stimulant drugs

Definition

Psychostimulants are drugs that substantially influence cognitive and affective functioning and behaviors. Effects are increased motivational desire, agitation, heightened vigilance, euphoria, hyperactivity, and

decreased sleepiness as well as appetite. Traditionally, mainly cocaine and amphetamines are considered psychostimulants. However, nicotine, caffeine, and even certain antidepressants could be included in this class. Methylphenidate, amphetamine (like dextro-amphetamine) and also pemoline – the latter was retracted from the market due to hepatotoxicity – are or have been widely used for the treatment of ►[attention deficit/hyperactivity disorder \(ADHD\)](#). Another psychostimulant indicated in ►[narcolepsy](#) and sleep apnea is modafinil.

Mechanism of Action

General Remarks

The final pathway of psychostimulants on the behavioral level is an increased mobilization of the normal fight/flight/fright reaction that is mediated by the biogenic amines epinephrine, norepinephrine, serotonin, and dopamine. The most widespread extraclinically used psychostimulant is ecstasy (3,4-methylenedioxymethamphetamine; MDMA), which also exhibits perceptual distortions due to 5-HT_{2A}-receptor agonism like lysergic acid diethylamide (LSD).

The main target of action of methylphenidate, the most widespread clinically used psychostimulant, is the dopamine transporter (DAT); its inhibition increases intrasynaptic dopamine concentrations. The subcortical dopamine system (mesolimbic and nigrostriatal parts) mediates the unconditioned and conditioned responses toward reinforcement.

Within the striatum, dopamine terminals have direct synaptic contacts with the spines of striatal neurons. These synaptic contacts appear to provide the anatomical substrate for both compartmental (synaptic) and volume (extrasynaptic) transmission. The DAT is located intra- or perisynaptically, suggesting that dopamine has a limited ability to escape the striatal synapse. Moreover, dopamine D2 and, to a lesser extent, D1 receptors are located postsynaptically from dopamine terminals, suggesting compartmental transmission. However, there is a significant cohort of D1 and D2 receptors that are not directly opposed to dopamine terminals, suggesting some component of volume transmission within the striatum. Therefore, tonic and phasic changes in dopamine transmission are critical in producing behavioral effects associated with the striatum. In contrast, in cerebral cortex, volume transmission appears to be more critical in mediating the effects of dopamine. DAT density is reduced in the ►[frontal cortex \(FC\)](#) relative to the striatum, and it is localized extrasynaptically. Moreover, D1-immunoreactivity within FC is virtually never opposed to tyrosine hydroxylase immunopositive terminals suggesting that the effect of dopamine on D1 receptors is by volume transmission. This hypothesis has direct relevance to the neurochemistry of

ADHD because cognitive functions known to be affected in this disorder, namely working memory and inhibitory control, are sensitive to manipulations of D1 receptor-mediated dopamine transmission [1]. Thus, the tonic component might be more critical for the behavioral functions of the FC.

Several classes of drugs modulate the firing rates or patterns of midbrain dopamine neurons by direct, monosynaptic, or indirect, polysynaptic, inputs to the cell bodies within the ventral mesencephalon (i.e., nicotine and opiates). In contrast, amphetamine, cocaine, and methylphenidate act at the level of the dopamine terminal interfering with normal processes of transmitter packaging, release, reuptake, and metabolism.

Methylphenidate, Amphetamine, and Cocaine

Methylphenidate like cocaine largely acts by blocking reuptake of monoamines into the presynaptic terminal. Methylphenidate administration produces an increase in the steady-state (tonic) levels of monoamines within the synaptic cleft. Thus, DAT inhibitors, such as methylphenidate, increase extracellular levels of monoamines. In contrast, they decrease the concentrations of the monoamine metabolites that depend upon monoamine oxidase (MAO), that is, HVA, but not catecholamine-*o*-methyltransferase (COMT), because reuptake by the transporter is required for the formation of these metabolites. By stimulating presynaptic autoreceptors, methylphenidate induced increase in dopamine transmission can also reduce monoamine synthesis, inhibit monoamine neuron firing and reduce subsequent phasic dopamine release.

The pharmacology of amphetamine is considerably more complex. It does not only block monoamine reuptake, but also directly inhibits the vesicular monoamine transporter, causing an increase in cytosolic but not vesicular dopamine concentration. This may lead to reverse transport of the amines via the membrane-bound transporters. Further mechanisms of amphetamine action are direct MAO inhibition and indirect release of both dopamine and serotonin in the striatum.

Mild increases in tonic dopamine release – as a consequence of the administration of both methylphenidate and amphetamine – could have important impact on subsequent phasic release by feedback mechanisms (lowering the concentration).

As pointed out before there are some major differences between the striatal and cortical dopamine terminals ([Table 1](#)).

Therefore, dopamine transporter inhibitors exhibit less effect in the FC. There, dopamine seems to be reuptaken by the norepinephrine transporter, which dopamine actually has a higher affinity for than norepinephrine itself.

Amphetamine administration produces a marked increase in cortical dopamine, norepinephrine, and

serotonin release that is impulse independent. Methylphenidate can produce significant increases in dopamine and norepinephrine release (Table 2).

Dopaminergic mechanisms within the ventral striatum (i.e., nucleus accumbens) subserve the ability of amphetamine and methylphenidate in low to moderate doses to increase locomotor activity. In contrast, very low dosages in animals seem to cause hypoactivity presumably by stimulation of autoreceptors, a finding that would be compatible with the clinical impression that methylphenidate might be useful in some patients with mania.

At low doses, both psychostimulants could theoretically stimulate tonic, extracellular levels of monoamines, and the small increase in steady state levels would produce feedback inhibition of further release by stimulating presynaptic autoreceptors. While this mechanism is clearly an important one for the normal regulation of monoamine neurotransmission, there is no direct evidence to support the notion that the doses used clinically to treat ADHD are low enough to have primarily presynaptic effects. However, alterations in phasic dopamine release could produce net reductions in dopamine release under putatively altered tonic dopaminergic conditions that might occur in ADHD and that might explain the beneficial effects of methylphenidate in ADHD.

Repeated intermittent exposure to stimulants can produce sensitization, where subsequent drug exposures produce increased behavioral and neurochemical responses. The ability of the drug and ultimately of related stimuli to elicit behavior may be increased with repeated administration or intake of the drug. Dopaminergic sensitization within the amygdala has also been found after repeated exposure to amphetamine and this

can enhance appetitive and aversive learning even after drug cessation.

Cocaine- and amphetamine-regulated transcript (CART) peptides are putative neurotransmitters are found in those dopaminergic brain regions. Stimulants seem to alter CART mRNA and peptide levels [2].

Besides the dopaminergic system, the noradrenergic nucleus, locus coeruleus (LC) may be another structure involved in the mode of action of psychostimulants. Electrophysiological recordings from this area demonstrate a relationship between behavior and response of the LC to targets versus distractors in an attention task. Baseline firing shows a constant increase paralleling the conditions from drowsiness to hyperarousal. In contrast, phasic responses are maximal in an optimal alert attentive state and minimal in drowsiness as well as hyperarousal that may be associated with poor cognitive performance due to high distractibility.

Finally, the cerebellum has recently become a focus of interest in the context of the pathophysiology of ADHD and as a possible target for psychostimulants since it is not only important for motor coordination but also for processing cognitive situations.

In summary, main structures involved in the action of psychostimulants can be divided into cortical (mainly prefrontal cortex) and subcortical (basal ganglia and related structures, LC and cerebellum) ones. Figure 1 gives a schematic overview of the connections between these structures, omitting the cerebellum due to lack of precise information.

Modafinil

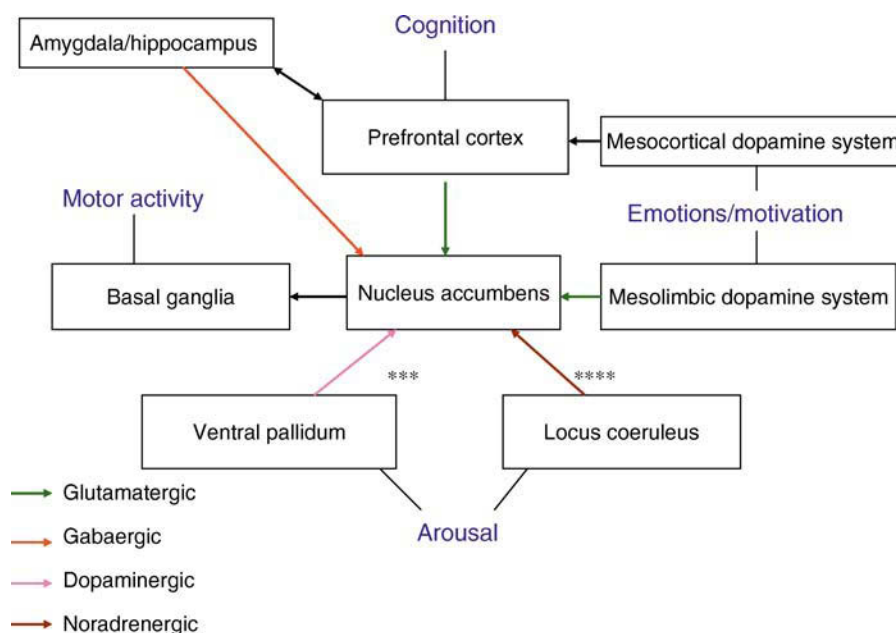
The mode of action of modafinil, a new arousal-promoting compound used in the treatment of sleepiness associated with narcolepsy, is not fully understood.

Psychostimulants. Table 1 Properties of cortical versus striatal dopamine system

	Cortical	Striatal
Synthesis-regulating autoreceptors	0	++
Impulse-regulating autoreceptors	0	++
DAT expression	0/+	++
DAT localization	Extrasynaptical	Synaptical

Psychostimulants. Table 2 Influence of the psychostimulants amphetamine, cocaine, and methylphenidate on the different biogenic amines

	Amphetamine	Cocaine	Methylphenidate
Dopamine	++	++	++
Norepinephrine	++	++	+
Serotonin	+	++	(+)



Psychostimulants. Figure 1 Nucleus accumbens as a major intersection of cognitive, motor and arousal processes.

It has been suggested that modafinil increases wakefulness by activating α_1 noradrenergic receptors or hypothalamic cells that contain the peptide ►[hypocretin](#) [3], or that it may act by modulating the GABAergic tone that might lead to an increased dopamine release in the nucleus accumbens. On the other hand, modafinil does not have any effect in DAT knockout mice.

Nicotine

Nicotine is the main psychoactive ingredient of tobacco and is responsible for the stimulant effects and abuse/addiction that may result from tobacco use. Cigarette smoking rapidly (in about 3 sec!) delivers pulses of nicotine into the bloodstream. Its initial effects are caused by its activation of ►[nicotinic acetylcholine \(nACh\) receptors](#). nACh receptors are ligand-gated ion-channels and pre- and postsynaptically located. Reinforcement depends on an intact mesolimbic dopamine system (VTA). nACh receptors on VTA dopamine neurons are normally activated by cholinergic innervation from the laterodorsal tegmental nucleus or the pedunculopontine nucleus.

Clinical Use

The main indication for certain psychostimulants is ADHD in children and adults [4]. Recent research shows that the clinical effect and benefit are dramatic even in adults. About 60% of adult patients receiving stimulant medication showed moderate-to-marked improvement, as compared with 10% of those receiving placebo. The core symptoms of hyperactivity,

inattention, mood lability, temper, disorganization, stress sensitivity, and impulsivity have been shown to respond to treatment with stimulant medications.

The characteristic behavioral effects of acute and chronic psychomotor stimulant drugs are locomotor activation, stereotypy, and conditioned reward and stimulus-reward learning. The most important brain regions involved in these effects are summarized in [Table 3](#).

Each of these processes depends upon increases in dopaminergic transmission within the striatum, and possibly, amygdala. Moreover, neurochemical actions within the FC may contribute to the ability to modulate some of these basic motivational processes. This data is based primarily on studies in rodents given systemic injections of moderate to high doses of stimulants, and it is not known whether lower doses applied orally would produce similar behavioral and neurochemical effects. Nevertheless, when given acutely or chronically to animals, stimulants appear to alter the neurochemistry of the striatum in such a way as to augment the control of behavior by conditioned or unconditioned stimuli associated with reinforcement processes. These effects may be consistent with the suggestion that amphetamine or methylphenidate may exert some of their beneficial clinical effects by augmenting conditioned reinforcement and stimulus-reward associations that could enhance aspects of task performance.

A simple decrease in striatal dopamine transmission produced by even low clinical doses of the drugs according to current data seems an untenable

hypothesis. However, if the tonic control of phasic release by dopamine is abnormally low in ADHD, then a high phasic dopamine response may be associated with ADHD and treatment efficacy. Moreover, alterations in corticolimbic tonic/phasic balance may also provide an explanation of why chronically administered psychostimulants in ADHD produce a behavioral profile and response associated with subcortical dopamine neuronal hyperactivity and cortical hypoactivity, though issues of dose and route of administration should be considered in this context.

Another theory for the action of stimulant drugs in ADHD involves effects on nonstriatal monoamine systems. Frontal cortical dopamine, norepinephrine, and serotonin are clearly important in cognitive functioning and impulse control. These neurotransmitters directly modulate reward-related behaviors associated with the striatal dopamine system. Moreover, the amygdala may be pharmacologically influenced leading to enhanced

associative learning and recall. Thus, the net behavioral effects of stimulant drugs may promote changes in reward-motivated behaviors and impulsivity, as well as neuromodulation of inhibitory control (FC), working memory, and incentive learning (amygdala).

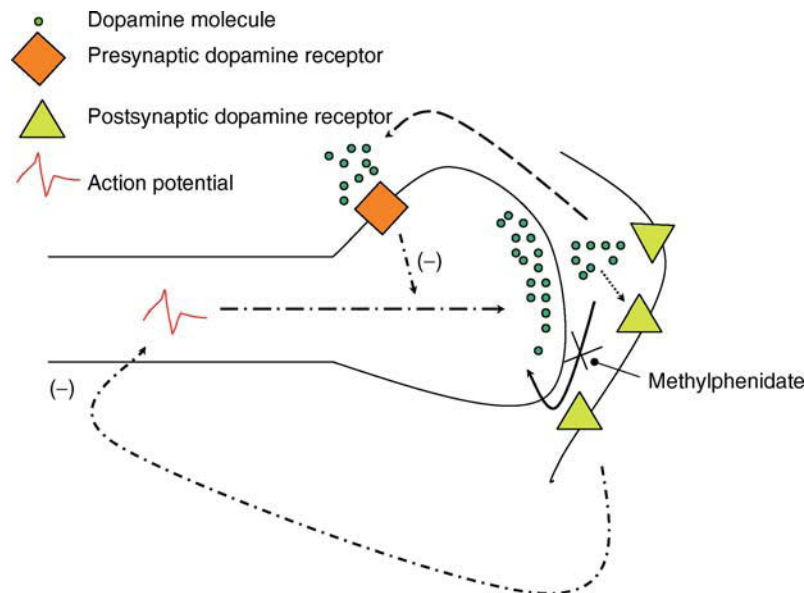
Dysfunction of cortical–subcortical dopamine systems is associated with an impaired inhibitory control after chronic drug administration.

Since there is some evidence that the dopaminergic system might also play an important role in the pathophysiology of depression, methylphenidate has also been successfully used as an augmentation strategy in the treatment of depressive disorders. Modafinil might be useful in this indication, too, besides its effect in narcolepsy and ADHD. It is usually well tolerated, although hyperkinesias and switch into mania might rarely occur.

Figure 2 [2] illustrates the acute and chronic actions of methylphenidate. By blocking DAT, methylphenidate

Psychostimulants. Table 3 Acute effects of psychostimulants and the brain regions that are mainly involved in these effects

Acute effects	Mainly involved brain region
Locomotion	Ventral striatum (i.e., nucleus accumbens)
Motor stereotypy	Dorsal striatum (i.e., caudate putamen)
Reinforcement	Ventral striatum
Conditioned reward	Ventral striatum/amygdala
Stimulus-reward learning	Amygdala



Psychostimulants. Figure 2 Dopamine molecules have two different possible targets. Both ways are initially increased by DAT inhibition caused by methylphenidate: pre- and postsynaptic dopamine receptors. Stimulation of postsynaptic receptors results in inhibition of presynaptic action potential generation. On the other hand, presynaptic receptor stimulation leads to a transmission inhibition of action potentials. Therefore, both mechanisms are responsible for a decrease in vesicular depletion of dopamine into the synaptic cleft (adapted from [2]).

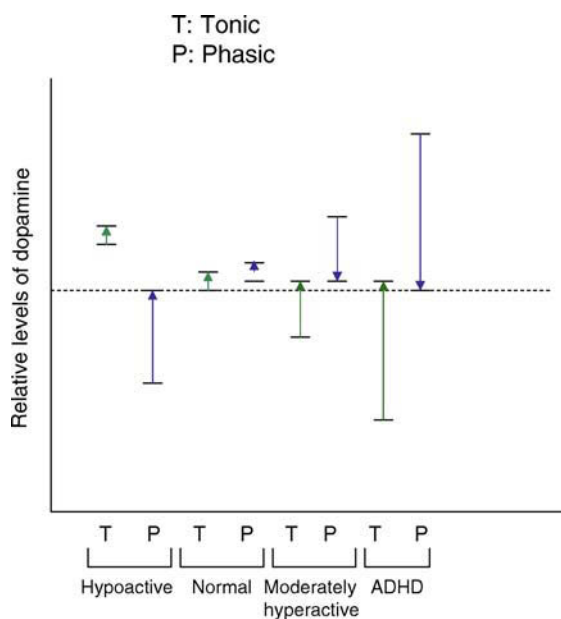
causes an accumulation of dopamine in the synaptic cleft. Although this may initially increase the stimulation of postsynaptic DA receptors, in the long term the consequence is rather a down-regulation of dopamine release. First, there is feedback inhibition of dopamine neuron firing to decreased spike-dependent dopamine release. Second, much larger quantities of dopamine are enabled to escape from the cleft and accumulate in the extrasynaptic space. Presynaptic receptors are stimulated and thus firing rate is reduced. The amount of phasic dopamine that can be released is subsequently diminished.

Moreover, no significant differences could be found between the efficacy of methylphenidate and amphetamine. Methylphenidate is faster metabolized and seems to be associated with fewer side effects regarding appetite loss and insomnia. From a clinical point of view, dosage and route of administration are the most important features influencing the spectrum of effects and side effects. ADHD drugs and cardiovascular risk (especially via increased blood pressure) has recently become a major issue with regard to sudden death. Summarizing, more selective and restricted use to clear-cut diagnoses are advocated.

An important clinical clue connected with the difference between tonic and phasic dopamine release is the so-called “rate dependence” of psychostimulant action. That means, it depends on the actual dopaminergic state (tonic and phasic) how an individual will react to psychostimulants. Figure 3 illustrates this rule by some examples [2]. The arrows represent the response of each component to methylphenidate for each of the classes of subjects tested, with the horizontal dashed line representing the baseline tonic and phasic levels present in control individuals. Summarizing, methylphenidate tends to normalize dopamine transmission regardless what the baseline rate is.

Despite their clinical use, psychostimulants are strongly reinforcing and their long-term use is linked to potential abuse and addiction, especially when they are rapidly administered. Nevertheless, long-term use is rather associated with emotional and motivational than with physical dependence. This is also true for cocaine and amphetamine. Methylphenidate might also be abused, although it is far less potent, possibly due to its specific mode of action (see above). Two drugs with low, if at all, abuse potential that are used in ADHD are atomoxetine [5], a noradrenalin reuptake inhibitor and bupropion, a dopamine- and noradrenalin reuptake inhibitor. By their mode of action they would rather fit into the group of antidepressants than psychostimulants.

Nicotine differs from cocaine in that it is powerfully reinforcing in the absence of subjective euphoria. The high incidence of cancerogenicity associated with long-term tobacco use is associated with compounds other than nicotine that are also contained in tobacco. Main



Psychostimulants. Figure 3 Effect of methylphenidate depending on baseline tonic (T) and phasic (P) dopamine levels. In a “normal” state only minimal changes are noted (which points to a rather low abuse potential). From a “hypoactive” state, methylphenidate increases both T and P levels. However, this is much more true for the strongly lowered P tone. In contrast, in moderately hyperactive states and ADHD, T levels are increased and P levels are decreased, respectively, correlating with the baseline levels (adapted from [2]).

short-term effects of nicotine are increased alertness, muscle relaxation, nausea, and increased psychomotor activation. Typical withdrawal symptoms include dysphoria, increased appetite, hyperventilation, and concentration difficulties. If the $\alpha 4\beta 2$ nicotinic partial agonist varenicline that is used for smoking cessation is also to be regarded as a stimulant drug still remains to be elucidated.

► Psychotomimetic Drugs ► Synaptic Transmission

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Psychotogenic

► Psychotomimetic Drugs

Psychotomimetic Drugs

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Synonyms

Hallucinogenic; Psychotogenic

Definition

Psychotomimetic drugs can be defined as chemical agents that reliably and dose-dependently induce a psychosis, often including hallucinations and delusions in normal individuals. Implicit in this term is a mimicking of naturally occurring psychosis.

Mechanism of Action

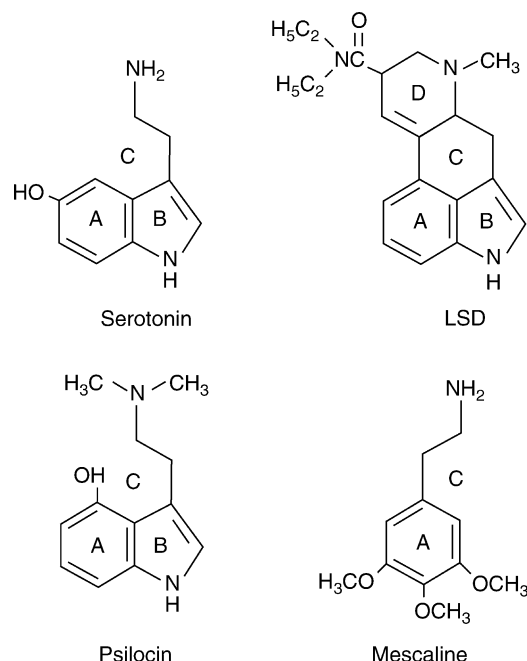
There are remarkable qualitative differences in the types of psychoses induced by different classes of psychotomimetic drugs. Some drugs cause such profound deficits in cognitive functioning that the resulting psychosis is associated with gross disorientation and confusion; a state sometimes called “dementia” or “organic psychosis.” A psychotomimetic effect of this kind is seen, for example, after high doses of atropine or scopolamine, drugs which block muscarinic cholinergic receptors. Drugs of this type serve to mimic or model Alzheimer’s disease and other severe brain disorders. There are other types of drugs that produce hallucinations and delusions without marked disorientation or confusion. Examples are ►**psychedelic hallucinogens** such as lysergic acid diethylamide (LSD) and mescaline and, in low doses, the ►**dissociative anesthetics** phencyclidine (PCP) and ketamine. This essay will focus upon the psychedelic hallucinogens and dissociative anesthetics, which are of

special interest because they are believed to model some important features of ►**schizophrenia** [1, 2].

For many years it was believed that the brain mechanisms underlying the effects of psychedelic hallucinogens and dissociative anesthetics were separate and distinct. Indeed, there has been considerable debate about which represents the best drug model of schizophrenia. However, recent data show that the two classes of psychotomimetic drugs share a common final pathway involving an increase in the release of the excitatory neurotransmitter ►**glutamate**.

Mechanism of Action of Psychedelic Hallucinogens

In the early 1950s, not long after the discovery of the powerful hallucinogenic properties of LSD, it was proposed that this drug interacted with a structurally related endogenous brain substance named ►**serotonin**. As seen in Fig. 1, LSD and serotonin (5-hydroxytryptamine; 5-HT) share an indolethylamine moiety, as do certain simpler indoleamine hallucinogens such as psilocin. On the other hand, the hallucinogen mescaline, despite having psychological or behavioral effects virtually identical to LSD, has a phenethylamine—rather than indoleamine—based structure. Nevertheless, mescaline does share certain other structural features



Psychotomimetic Drugs. Figure 1 Chemical structures of *serotonin* (5-hydroxytryptamine; 5-HT), the ergoline hallucinogen LSD (lysergic acid diethylamide), the simple indoleamine hallucinogen psilocin, and the phenethylamine hallucinogen mescaline. The letters A–D denote the four rings of LSD which are shared in varying aspects by the other hallucinogens as well as serotonin.

with LSD (Fig. 1), consistent with the hypotheses that these two drugs have a common site of action. In the mid-1980s it was shown that the hallucinogenic potency of indoleamine and phenethylamine hallucinogens could be predicted by their affinity for a subtype of serotonin receptor, the 5-HT_{2A} receptor [3]. The recognition of an association between hallucinogens and the 5-HT_{2A} receptor has formed the foundation for contemporary research in the field. Presently there is much evidence from biochemical, electrophysiological, and animal as well as human behavioral studies that the effects of hallucinogens are mediated through a partial agonist action at 5-HT₂ receptors, particularly of the 5-HT_{2A} subtype [4].

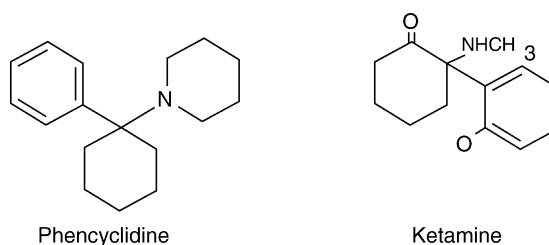
The effects of hallucinogens on an array of complex integrative processes such as cognition, perception and mood suggest the involvement of the cerebral cortex. In support of this idea, histochemical mapping by receptor autoradiography, mRNA in situ hybridization, and immunocytochemistry shows a high expression of 5-HT_{2A} receptors in the cerebral cortex, particularly within the apical dendrites of cortical ►pyramidal cells. At an electron microscopic level, an association has been observed between 5-HT_{2A} immunoreactivity and the postsynaptic density of excitatory synapses in the neocortex.

The high density of 5-HT_{2A} receptors in the cerebral cortex has stimulated study of the physiological role of this receptor in that region of brain [4]. A striking effect of serotonin in the cerebral cortex is to increase the release of ►glutamate onto layer V ►pyramidal cells of neocortex, as measured by electrophysiological recording of “spontaneous” excitatory postsynaptic potentials (►EPSPs). In vitro studies in brain slices show that EPSPs are induced by serotonin through a focal action at the apical dendrites of pyramidal cells. This effect is blocked by low concentrations of highly selective 5-HT_{2A} antagonists and is virtually absent in mice where there has been a genetic deletion of the 5-HT_{2A} receptor. While a serotonin-induced increase in ►EPSPs occurs throughout the neocortex, this effect is most pronounced in frontal areas such as the medial prefrontal cortex where there is an increased density of 5-HT_{2A} receptors as compared to more posterior regions. It has been postulated that the effect of serotonin is mediated indirectly by a ►retrograde messenger since 5-HT_{2A} receptors have a predominantly postsynaptic localization. Recently, it has been found that LSD and other hallucinogenic drugs, while having relatively low efficacy in inducing spontaneous ►EPSPs, dramatically increase the probability of occurrence of a late component of EPSPs evoked by electrical stimulation of the subcortical white matter. In contrast, serotonin itself usually does not promote the late component of electrically evoked EPSPs, probably due to opposing actions at 5-HT₁ or other non-5-HT_{2A}

receptors. It has been proposed that excessive induction of late EPSPs may underlie the disruptive effects of hallucinogens upon cortical function. The opposition by non-5-HT_{2A} receptors of this effect may explain why treatments that elevate endogenous serotonin (e.g., monoamine oxidase inhibitors or selective serotonin uptake blockers) are not hallucinogenic and may in fact attenuate the subjective effects of hallucinogens in humans.

Mechanism of Action of Dissociative Anesthetics

The most commonly used (or abused) drugs in this category are the structurally related drugs, PCP and ketamine (Fig. 2). At high doses these drugs produce a delirium-like state as seen in stage I anesthesia. Lower, subanesthetic doses are capable of producing a psychotomimetic state in normal subjects that stops short of frank delirium. Both PCP and ketamine are known to act as non-competitive antagonists of the ►NMDA receptor; a subtype of ionotropic ►glutamate receptor [5]. The NMDA receptor belongs to the general class of ligand-gated nonselective cation channels and is distinguished within this group by its high permeability for calcium in addition to sodium and potassium ions. Because of its high calcium permeability, the NMDA receptor has been linked to various intracellular calcium signaling pathways. Another notable characteristic of NMDA receptor channels is blockade by magnesium ions under resting or hyperpolarized conditions, a block that is relieved under excitatory, depolarizing conditions. As suggested by the noncompetitive nature of the blockade, these drugs do not act directly at the glutamate ligand binding site, but rather interact with a separate site within the pore of the open channel, hence their designation as “open channel” blockers. While other actions also have been described, it is generally believed that the primary mechanism by which PCP and ketamine produce their adverse behavioral effects is through blockade of NMDA receptors. The net effect of these drugs has sometimes been thought of in terms of a general



Psychotomimetic Drugs. Figure 2 Chemical structures of the dissociative anesthetics phencyclidine (PCP) and ketamine. Both are arylcycloalkylamine derivatives that are open channel blockers of the NMDA channel.

impairment of glutamatergic transmission. However, it should be noted that these drugs do not block non-NMDA glutamate receptors, including other ionotropic glutamate receptors (i.e., AMPA and kainate) and all three major groups of metabotropic glutamate receptors. Recently, it has been shown that many of the effects of NMDA antagonists may be mediated through increased release of glutamate in prefrontal cortex and other regions, causing excessive activation of non-NMDA receptors. Suppression of excessive glutamate release ameliorates many of the resulting adverse behavioral effects. The mechanism by which NMDA antagonists increase glutamate release appears to be distinct from that of 5-HT_{2A} agonists since local application of phencyclidine to brain slices, in contrast to local serotonin application, does not result in an increase in EPSCs in layer V pyramidal cells of prefrontal cortex.

Conclusions

This essay has highlighted both differences and similarities in the mechanism of action of the psychedelic hallucinogens and ►dissociative anesthetics. The initial sites at which these two classes of psychotomimetics act are quite different: the psychedelic hallucinogens acting via 5-HT_{2A} receptors and dissociative anesthetics through blockade of NMDA receptors. However, recent evidence reveals intriguing common features downstream from the initial receptors. Most notably, both hallucinogens and dissociative anesthetics indirectly produce an enhancement of glutamate release in the cerebral cortex. However, it would not be expected that the effects of the two classes of drugs would be identical since in the case of the psychedelic hallucinogens, NMDA receptors are not blocked. Blockade of NMDA receptors may account for the progressive disorientation or delirium seen clinically with near-anesthetic doses of the phencyclidine/ketamine class of drugs, effects that are not characteristic of the psychedelic hallucinogens. Nevertheless, evidence for an increase in glutamate transmission for both psychedelic hallucinogens and dissociative anesthetics points to a convergence upon a final common glutamatergic pathway to account for overlapping aspects of their psychotomimetic effects.

►Psychostimulants

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PT

►Prothrombin Time

PTB Domain

Synonyms

Phosphotyrosine-Binding

Definition

Protein-protein interaction domain that recognizes short sequences containing a phosphotyrosine. Hydrophobic residues N-terminal to the phosphotyrosine residue provide distinction from SH2 domains. Particularly important in assembling protein complexes at activated receptors.

►Adaptor Proteins

PTEN

PTEN is a phosphatase, which is a product of a tumor suppressor gene. This phosphatase has an unusual broad specificity and can remove phosphate groups attached to serine, threonine, and tyrosine residues. It is believed that its ability to dephosphorylate phosphatidylinositol (PI) 3,4,5-triphosphate, the product of PI-3 kinase, is responsible for its tumor suppressor effects.

- Phosphatases
- Phospholipid Kinases
- Phospholipid Phosphatases
- Protein Phosphatases

PTM

► Post-translational Modification

Pulmonary Emphysema

► Chronic Obstructive Pulmonary Disease

Pulmonary Hypertension

Primary pulmonary hypertension is a disease of unclear etiology that is characterized by abnormally high mean pulmonary arterial pressures, in the absence of a demonstrable cause. A wide variety of pulmonary and cardiac diseases can lead to secondary pulmonary hypertension.

Purinergic System

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Definition

The purinergic system is a signalling system, where the purine nucleotides, ►ATP (Adenosine 5'-triphosphate) and ADP (Adenosine diphosphate), and the nucleoside, ►adenosine, act as extracellular messengers. This concept, which was first proposed over 30 years ago [1], met with considerable resistance for many years, because ATP had been established as an intracellular energy source involved in various metabolic cycles and it was thought that such a ubiquitous molecule was unlikely to be involved in selective extracellular signalling. However, ATP was one of the first molecules to appear in biological evolution so that it is not really surprising that it should have been utilized early for extracellular, as well as intracellular, purposes. The existence of potent extracellular enzymes that regulate the amount of ATP

and adenosine available for signalling, also provides support that ATP has extracellular actions.

Basic Characteristics

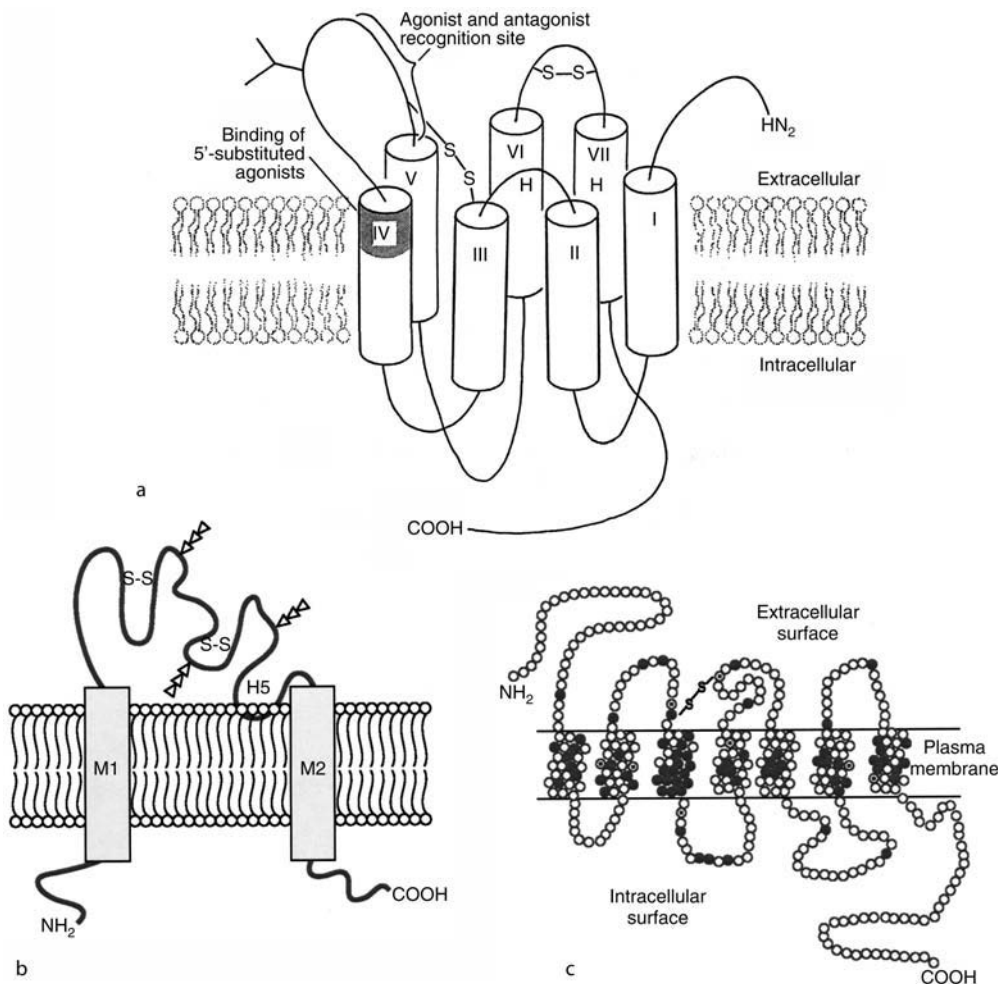
Purinoreceptor Subtypes

Implicit in purinergic signalling is the presence of receptors for ATP [2]. A basis for distinguishing adenosine receptors (P1), from ATP/ADP receptors (P2), was proposed in 1978. This helped resolve some of the earlier ambiguous reports, which were complicated by the breakdown of ATP to adenosine by ectoenzymes, so that some of the actions of ATP were directly on P2 receptors while others were due to indirect action via P1 receptors. Four subtypes of P1 receptors have been cloned, namely, A₁, A_{2A}, A_{2B} and A₃ (Fig. 1a). P2 receptors belong to two families based on molecular structure and second messenger systems, namely P2X ionotropic ligand-gated ion channel receptors and P2Y metabotropic G protein-coupled receptors. This framework allows for a logical expansion as new receptors are identified. There are currently seven subtypes of P2X receptors and eight subtypes of P2Y receptors identified and characterized in mammals (Table 1). P2X receptors are characterized by two transmembrane domains, short intracellular N- and C-termini and an extensive extracellular loop with conservation of ten cysteines (Fig. 1b). Broadly, P2X₁ receptors are strongly represented in smooth muscle, P2X₂, P2X₄ and P2X₆ receptors in the central nervous system, P2X₃ receptors on sensory neurones, P2X₅ receptors are associated with cell proliferation and differentiation and P2X₇ receptors with cell death. The ion pores appear to consist of three subunits forming homomultimers or heteromultimers, including P2X_{1/2}, P2X_{1/4}, P2X_{1/5}, P2X_{2/3}, P2X_{2/6} and P2X_{4/6}. P2Y receptors, in common with other G protein-coupled receptors, have seven transmembrane domains, an extracellular N, and intracellular C-terminus (Fig. 1c). P2Y₁ receptors are ADP-selective in mammals and 2-methylthioADP and MRS 2179 are selective agonists and antagonists, respectively. At P2Y₂ and P2Y₄ receptors in the rat, ATP and UTP are equipotent, but the two receptors can be distinguished with antagonists. P2Y₆ is UDP-selective. P2Y₁₁ is unusual in that there are two transduction pathways, adenylate cyclase as well as inositol triphosphate, which is the second messenger system used by the majority of the P2Y receptors. The P2Y₁₂ receptor is found on platelets. P2Y₁₃ is ADP-selective and acts via adenylate cyclase, while P2Y₁₄ receptors are selective for UDP-glucose and UDP-galactose.

Physiology

Short-Term Neuronal Signalling

There was early evidence that ATP was a ►neurotransmitter in non-adrenergic, non-cholinergic (NANC) nerves supplying the gut and bladder. There is now



Purinergic System. Figure 1 Membrane receptors for extracellular ATP and adenosine. P1 family of receptors for extracellular adenosine are G protein-coupled receptors signalling by inhibiting or activating adenylate cyclase (a). The P2 family of receptors bind extracellular ATP or ADP, and is comprised of two types of receptors (P2X and P2Y). The P2X family of receptors are ligand-gated ion channels (b), and the P2Y family are G protein-coupled receptors (c). (a, reproduced from [2] with permission from the American Society for Pharmacology and Experimental Therapeutics; b, from Brake AJ, Wagenbach MJ, Julius D (1994) New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. *Nature* 371:519-523, reproduced with permission from Nature; c modified from Barnard EA, Burnstock G, Webb TE (1994) G protein-coupled receptors for ATP and other nucleotides: a new receptor family. *Trends Pharmacol Sci* 15:67-70, reproduced with permission from Elsevier).

supporting evidence that ATP is a cotransmitter in many nerve types [1], probably reflecting the primitive nature of purinergic signalling. Thus, there is now evidence for ATP as a cotransmitter with:

- Noradrenaline (NA) and neuropeptide Y (NPY) in sympathetic nerves
- ATP with acetylcholine and vasoactive intestinal peptide in some parasympathetic nerves
- ATP with nitric oxide and vasoactive intestinal peptide in enteric NANC inhibitory nerves
- ATP with calcitonin gene-related peptide and substance P in sensory-motor nerves

There is also evidence for ATP as a cotransmitter with γ -aminobutyric acid or with glutamate, serotonin, NA or dopamine in nerves in the central nervous system.

In sympathetically innervated tissues, such as vas deferens or blood vessels, ATP produces fast responses mediated by P2X receptors followed by a slower component mediated by G protein-coupled α -adrenoceptors (Fig. 2); NPY usually acts as a pre- or postjunctional modulator of the release and/or action of NA and ATP. Similarly, for parasympathetic nerves supplying the urinary bladder, ATP provokes a fast, short-lasting twitch response via P2X receptors, whereas the slower component is mediated by G

Purinerger System. Table 1 Receptor subtypes for purines and pyrimidines: distribution, agonists, antagonists and transduction mechanisms (updated and reprinted from Burnstock G (2003) Introduction: ATP and its metabolites as potent extracellular agonists. In: Schwiebert EM (ed) Current topics in membranes, vol 54. Purinerger receptors and signalling. Academic Press, San Diego, pp 1–27, with permission from Elsevier)

Receptor		Main distribution	Agonists	Antagonists	Transduction mechanisms
P1 (adeno-sine)	A ₁	Brain, spinal cord, testis, heart, autonomic nerve terminals	CCPA, CPA, S-ENBA, CVT-510	DPCPX, N-0840, MRS1754, N-0840, WRC-0571	G _{i/o} ↓cAMP
	A _{2A}	Brain, heart, lungs, spleen	CGS 21680, HE-NECA, CVT-3146	KF17837, SCH58261, ZM241385, KW 6002	G _S ↑cAMP
	A _{2B}	Large intestine, bladder	NECA (non-selective)	Enprofylline, MRE2029-F20, MRS17541, MRS 1706	G _S ↑cAMP
	A ₃	Lung, liver, brain, testis, heart	IB-MECA, 2-CI-IB-MECA, DBXRM, VT160	MRS1220, L-268605, MRS1191, MRS1523, VUF8504	G _{i/o} G _{q/11} ↓cAMP ↑IP ₃
P2X	P2X ₁	Smooth muscle, platelets, cerebellum, dorsal horn spinal neurons	α,β-meATP = ATP = 2-MeSATP, L-β,γ-meATP (rapid desensitization)	TNP-ATP, Ip ₅ I, NF023, NF449	Intrinsic cation channel (Ca ²⁺ and Na ⁺)
	P2X ₂	Smooth muscle, CNS, retina, chromaffin cells, autonomic and sensory ganglia	ATP ≥ ATPγS ≥ 2-MeSATP > > α,β-meATP (pH + zinc sensitive)	Suramin, isoPPADS, RB2, NF770, NF279	Intrinsic ion channel (particularly Ca ²⁺)
	P2X ₃	Sensory neurones, NTS, some sympathetic neurons	2-MeSATP ≥ ATP ≥ α,β-meATP ≥ Ap ₄ A (rapid desensitization)	TNP-ATP, PPADS, A317491, NF110, Ip ₅ I, phenol red	Intrinsic cation channel
	P2X ₄	CNS, testis, colon	ATP > > α,β-meATP, CTP ivermectin potentiation	TNP-ATP (weak), BBG (weak), phenolphthalein	Intrinsic ion channel (especially Ca ²⁺)
	P2X ₅	Proliferating cells in skin, gut, bladder, thymus, spinal cord	ATP > > α,β-meATP, ATPγS	Suramin, PPADS, BBG	Intrinsic ion channel
	P2X ₆	CNS, motor neurons in spinal cord	– (does not function as homomultimer)	–	Intrinsic ion channel
	P2X ₇	Apoptotic cells in, for example, immune cells, pancreas, skin	BzATP > ATP ≥ 2-MeSATP > > α,β-meATP	KN62, KN04, MRS2427, O-ATP Coomassie brilliant blue G	Intrinsic cation channel and a large pore with prolonged activation
P2Y	P2Y ₁	Epithelial and endothelial cells, platelets, immune cells, osteoclasts	2-MeSADP = ADPβS > 2-MeSATP = ADP > ATP, MRS2365	MRS2179, MRS2500, MRS2279, PIT	G _q /G ₁₁ ; PLC-β activation

Purinergic System. Table 1 Receptor subtypes for purines and pyrimidines: distribution, agonists, antagonists and transduction mechanisms (updated and reprinted from Burnstock G (2003) Introduction: ATP and its metabolites as potent extracellular agonists. In: Schwiebert EM (ed) Current topics in membranes, vol 54. Purinergic receptors and signalling. Academic Press, San Diego, pp 1–27, with permission from Elsevier) (Continued)

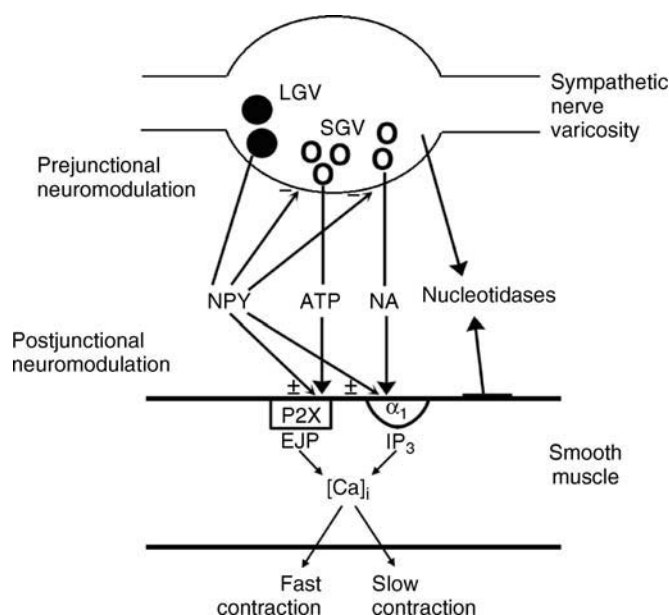
Receptor		Main distribution	Agonists	Antagonists	Transduction mechanisms
	P2Y ₂	Immune cells, epithelial and endothelial cells, kidney tubules, osteoblasts	UTP = ATP, UTPγS, INS 37217, INS 365	Suramin > RB2, AR-C126313	G _q /G ₁₁ and possibly G _i ; PLC-β activation
	P2Y ₄	Endothelial cells	UTP ≥ ATP, UTPγS, INS 37217	RB2 > suramin	G _q /G ₁₁ and possibly G _i ; PLC-β activation
	P2Y ₆	Some epithelial cells, placenta, T cells, thymus	UDP > UTP > > ATP, UDPβS, IDP	MRS2578	G _q /G ₁₁ ; PLC-β activation
	P2Y ₁₁	Spleen, intestine, granulocytes	AR-C67085MX > BzATP ≥ ATPγS > ATP	Suramin > RB2, NF157, 5'-AMPS	G _q /G ₁₁ and G _S ; PLC-β activation
	P2Y ₁₂	Platelets, glial cells	2-MeSADP ≥ ADP > > ATP	CT50547, AR-C69931MX, INS49266, AZD6140, PSB0413, ARL66096, 2-Me-SAMP	G _{i/o} ; inhibition of adenylate cyclase
	P2Y ₁₃	Spleen, brain, lymph nodes, bone marrow	ADP = 2-MeSADP > > ATP = 2-Me-SATP	MRS2211, 2-Me-SAMP	G _{i/o}
	P2Y ₁₄	Placenta, adipose tissue, stomach, intestine, discrete brain regions	UDP glucose = UDP-galactose	–	G _q /G ₁₁

Ap₄A, diadenosine tetraphosphate; BBG, Brilliant blue green; BzATP, 2'-&3'-O-(4-benzoyl-benzoyl)-ATP; cAMP, cyclic AMP; CCPA, chlorocyclopentyl adenosine; CPA, cyclopentyl adenosine; CTP, cytosine triphosphate; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; IP₃, inosine triphosphate; Ip₅I, diinosine pentaphosphate; α,β-meATP, α,β-methylene ATP; β,γ-meATP, β,γ-methylene ATP; 2-MeSADP, 2-methylthio ADP; 2-MeSAMP, 2-methylthio AMP; 2-MeSATP, 2-methylthio ATP; NECA, 5'-N-ethylcarboxamido adenosine; PPADS, pyridoxal-phosphate-6-azophenyl-2', 4'-disulfonic acid; PLC, phospholipase C; RB2, reactive blue 2; TNP-ATP, 2',3'-O-(2,4,6-trinitrophenyl) ATP.

protein-coupled muscarinic receptors. In the gut, ATP released from NANC inhibitory nerves produces the fastest response, nitric oxide gives a less rapid response, and vasoactive intestinal peptide produces slow tonic relaxations. In all cases of ►cotransmission, there are considerable differences in the proportion of the cotransmitters in nerves supplying different regions of the gut or vasculature, in different developmental or pathophysiological conditions and between species.

The first clear evidence for nerve-nerve purinergic ►synaptic transmission was in 1992, when it was

shown that excitatory postsynaptic potentials in the celiac ganglion and in the medial habenula in the brain were reversibly antagonized by suramin, a P2 receptor antagonist. Since then, there have been many articles describing either the distribution of various P2 receptor subtypes in the brain and spinal cord or electrophysiological studies of the effects of purines in brain slices, isolated nerves and glial cells. Synaptic transmission has also been found in the myenteric plexus and in various sensory, sympathetic and pelvic ganglia.



Purinergic System. Figure 2 Schematic of sympathetic cotransmission. ATP and NA released from small granular vesicles (SGV) act on P2X and α_1 receptors on smooth muscle, respectively. ATP acting on inotropic P2X receptors evokes excitatory junction potentials (EJPs), increase in intracellular calcium ($[Ca^{2+}]_i$) and fast contraction; while occupation of metabotropic α_1 -adrenoceptors leads to production of inositol triphosphate (IP_3), increase in $[Ca^{2+}]_i$ and slow contraction. Neuropeptide Y (NPY) stored in large granular vesicles (LGV) acts after release both as a prejunctional inhibitory modulator of release of ATP and NA and as a postjunctional modulatory potentiator of the actions of ATP and NA. Soluble nucleotidases are released from nerve varicosities, and are also present as ectonucleotidases. (Reproduced from Burnstock G (2007) Neurotransmission, neuromodulation: cotransmission. In: Squire LR (ed) New encyclopaedia of neuroscience. Elsevier, The Netherlands (In Press), with permission from Elsevier).

Short-Term Non-Neuronal Signalling

There are many examples of purinoceptor-mediated responses in non-neuronal cell types [3]. ► **Endothelial cells**, which express P2Y₁, P2Y₂ and probably P2Y₆ and P2X₄ receptors, when occupied, release nitric oxide leading to vasodilatation (Fig. 3). The more recent discovery of P2X receptors in endothelial cells suggests a role regulating gap and tight junctions involved in permeability and in cell adhesion. P2Y₁ receptors in pancreatic β -cells have been shown to be involved in insulin secretion, and P2Y₂ receptors are present on hepatocytes. P2Y₁₂, P2X₁ and P2Y₁ receptors are expressed in platelets and P2Y₁ and P2Y₂ receptors on non-myelinating and myelinating Schwann cells, respectively. Purinergic receptors are also involved in signalling to endocrine cells, leading to hormone secretion and in neuron-glia cell interactions.

Long-Term (Trophic) Signalling

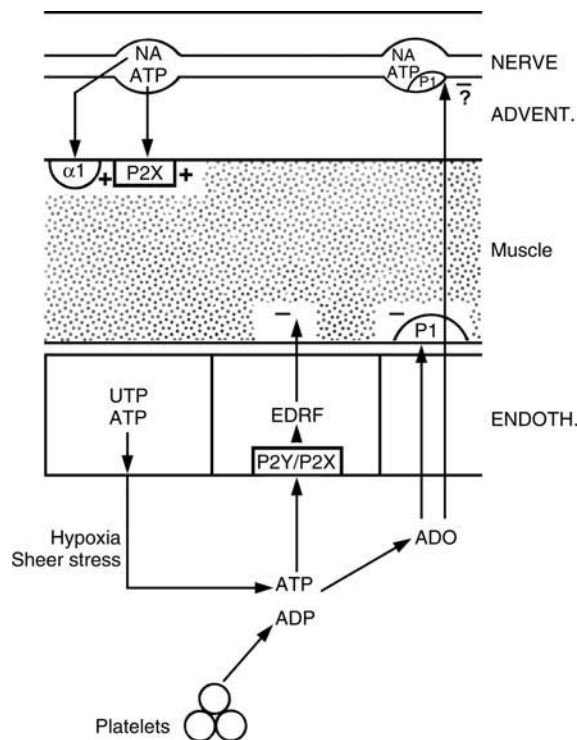
Purinergic signalling is also concerned with long-term events, such as cell proliferation, migration, differentiation and death associated with development and regeneration [1, 3, 4]. For example, α,β -methylene ATP produces proliferation of glial cells, whereas adenosine inhibits proliferation. A p2y8 receptor was

cloned from the frog embryo, which appears to be involved in the development of the neural plate. P2Y₁ receptors seem to have a role in cartilage development in limb buds and in development of the mesonephros. P2X₅ and P2X₆ receptors have been implicated in the development of chick skeletal muscle. In recent studies of purinoceptor expression in developing mouse myotubes, there was progressive expression of P2X₅, P2X₆ and P2X₂ receptors. The P2X₁ receptor is prominent in contractile smooth muscle phenotype, but is absent in the synthetic smooth muscle phenotype grown in culture, while P2Y receptor expression is substantially increased. There are several reports showing that P2X and P2Y receptors in ► **osteoclasts** and ► **osteoblasts** are involved in bone development and remodelling.

Pathophysiology and Therapeutic Potential

There is increasing interest in the therapeutic potential of purinergic compounds in relation to both P1 and P2 receptors [4]. A number of purine-related compounds have been patented.

It is well established that the ► **autonomic nervous system** shows marked plasticity. The expression of cotransmitters and receptors shows dramatic changes during development and ageing, in nerves that remain



Purinergic System. Figure 3 A schematic representation of the interactions of ATP released from perivascular nerves and from the endothelium (ENDOTH.). ATP and UTP are released from endothelial cells during changes in blood flow (sheer stress) and hypoxia to act on endothelial P2Y and P2X receptors leading to production of EDRF (nitric oxide) and subsequent vasodilatation (-). In contrast, ATP released as a cotransmitter with noradrenaline (NA) from perivascular sympathetic nerves at the adventitia (ADVENT.)/muscle border produces vasoconstriction (+) via P2X receptors on the muscle cells. Adenosine (ADO), resulting from rapid breakdown of ATP by ectoenzymes, produces vasodilatation by direct action on the muscle via P1 receptors and acts on the perivascular nerve terminal varicosities to inhibit transmitter release. (Modified from Burnstock G (1987) Local control of blood pressure by purines. *Blood Vessels* 24:156–160, reproduced with permission from Karger AG).

after trauma or surgery and in disease conditions. There are now a number of examples where the purinergic component of cotransmission is increased in pathological conditions [4]. Purinergic nerve-mediated contractions of the human bladder are increased up to 40% in pathophysiological conditions such as interstitial cystitis, outflow obstruction and probably also neurogenic bladder. ATP plays a significantly greater cotransmitter role in sympathetic nerves supplying hypertensive blood vessels. Upregulation of P2X₁ and P2Y₂ receptor mRNA

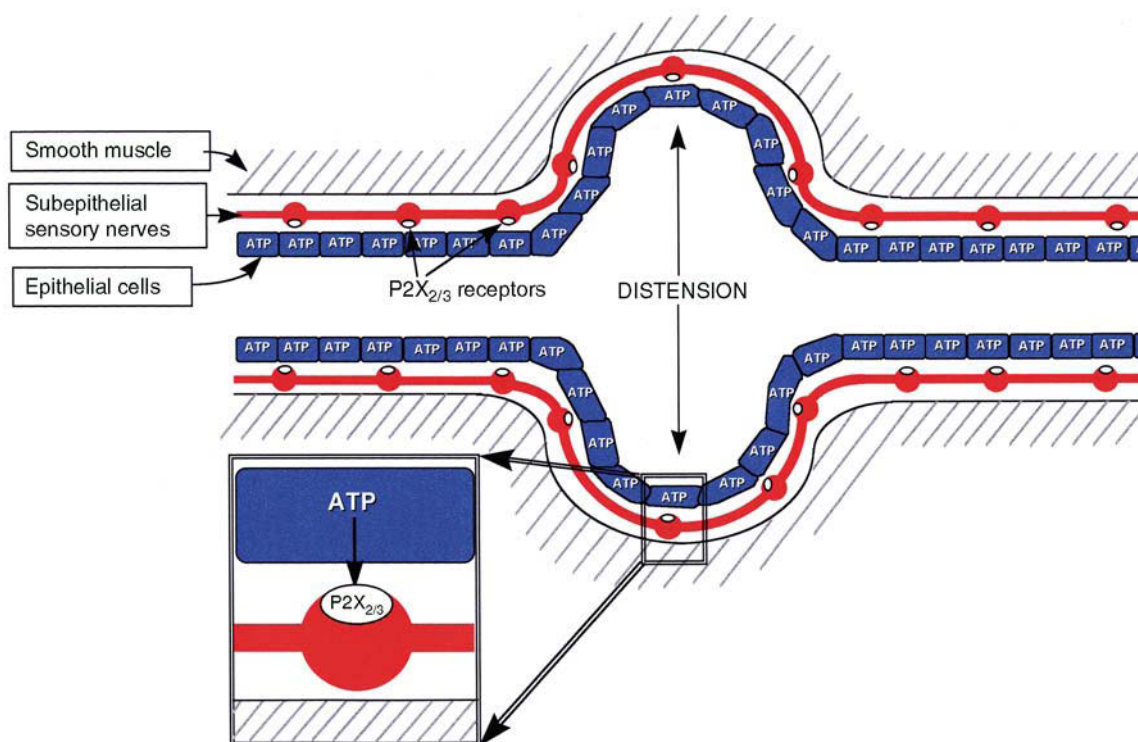
in hearts of rats with congestive heart failure has been reported. Adenosine modulates long-term synaptic plasticity in the hippocampus; it attenuates long-term potentiation (LTP); P1 receptor antagonists facilitate LTP. It is suggested that adenosine-related compounds might prove helpful in the treatment of memory disorders and intellectual performance related to caffeine intake.

A new hypothesis for purinergic **▶mechanosensory transduction** in visceral organs involved in the initiation of pain has been proposed. It is suggested that distension of tubes (such as the ureter, salivary duct and gut), and sacs (such as urinary and gall bladder), leads to the release of ATP from the lining epithelial cells that diffuses to subepithelial sensory nerves expressing P2X₃ and/or P2X_{2/3} nociceptive receptors, which mediate messages to pain centres in the central nervous system [5] (Fig. 4). Recording in P2X₃ knockout mice, has shown that the micturition reflex is impaired and that responses of sensory fibres to P2X₃ agonists are gone, suggesting that P2X₃ receptors on sensory nerves in the bladder have a physiological as well as a nociceptive role.

P1 (adenosine) receptors were explored as therapeutic targets before P2 receptors. Adenosine was identified early and is in current use to treat **▶supraventricular tachycardia**. A_{2A} receptor antagonists are being investigated for the treatment of **▶Parkinson's disease** and patents have been lodged for the application of P1 receptor subtype agonists and antagonists for myocardial ischaemia and reperfusion injury, cerebral ischaemia, stroke, intermittent claudication and renal insufficiency.

Purinergic receptors have a strong presence in bone cells. P2X and P2Y receptors are present on osteoclasts, with P2Y receptors only being present on osteoblasts. ATP, but not adenosine, stimulates the formation of osteoclasts and their resorptive actions in vitro and can inhibit osteoblast-dependent bone formation. A recent study has shown that very low (nM) concentrations of ADP acting through P2Y₁ receptors turn on osteoclast activity. Modulation of P2 receptor function may have potential in the treatment of osteoporosis. The anticancer activity of adenine nucleotides was first described in 1983 and since then, intraperitoneal injection of ATP into tumour-bearing mice has resulted in significant anticancer activity against several fast-growing aggressive carcinomas.

Purinergic signalling is important in the special senses. For example, P2Y₂ receptor activation increases salt, water and mucus secretion in the eye and thus represents a potential treatment for dry eye disease. P2 receptor agonists have greater efficacy in reducing intraocular pressure than cholinergic and adrenergic agents, raising possibilities for novel treatment of glaucoma. It has been



Purinergic System. Figure 4 Schematic representation of the hypothesis for purinergic mechanosensory transduction in tubes (e.g. ureter, vagina, salivary and bile duct and gut) and sacs (e.g. urinary and gall bladders, and lung). It is proposed that distension leads to release of ATP from the epithelium lining the tube or sac, which then acts on $P2X_{2/3}$ receptors on subepithelial sensory nerves to convey sensory (nociceptive) information to the CNS. (From Burnstock G, Wood JN (1996) Purinergic receptors: their role in nociception and primary afferent neurotransmission. *Curr Opin Neurobiol* 6:526–532, with permission from Blackwell Publishing).

suggested that ATP may regulate fluid homeostasis, cochlear blood flow, hearing sensitivity and development, and thus may be useful in the treatment of Ménière's disease, tinnitus and sensorineural deafness.

There have been very promising recent developments concerning purinergic drugs aimed at treating ►thrombosis. Clopidogrel and ticlopidine are antagonists of the $P2Y_{12}$ receptor and appear to reduce the risks of recurrent strokes and heart attacks, especially when combined with aspirin. Further therapeutic targets include: chronic renal failure, congestive heart failure, hypertension, stroke, angina, asthma, chronic obstructive pulmonary disease, epilepsy, sleep apnea, diabetes, inflammation, erectile function and wound healing.

Drugs

See Table 1.

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Purinoceptor

A purinoceptor is a cell surface receptor for the purinergic nucleotides ATP and ADP and for the purine nucleotide, adenosine.

►Purinergic System

PXR

Pregnane X Receptor, Nuclear Receptor Regulation of Drug-metabolizing P450-Enzymes.

Pyknosis

Pyknosis refers to a degenerative (thickening) process in a cell in which the nucleus shrinks in size and chromatin condenses to a solid mass that has no defined structure.

► Neurodegeneration

Pyramidal Cell

Pyramidal neurons are the principal long-projecting cells of the cerebral cortex and hippocampus. They are so named because of the characteristic large apical dendrite, giving them a pyramidal shape.

► Psychotomimetic Drugs

Pyrexia

► Fever

Pyrogenic Cytokines

A class of immunoregulatory polypeptides produced by various cells types, but predominantly by mononuclear phagocytes activated by signals provided by exogenous pyrogens. These peptides act as endogenous pyrogens.

► Fever

PYY₃₋₃₆

PYY₃₋₃₆ is an endogenously occurring fragment of *peptide YY*, which is generated upon cleavage by the enzyme dipeptidylpeptidase IV, also known as *CD26*. In contrast to *peptide YY*, PYY₃₋₃₆ is selective for the Y₂ and Y₅ relative to the Y₁ subtype of NPY receptors.

► Neuropeptide Y

QA/QC Control Metrics

Quality Assurance/Quality Control parameter and metrics to ensure data reproducibility, e.g. the establishment of calibrated RNA samples and reference datasets to objectively assess the performance of different microarray platforms (see also MAQC project: ►<http://www.nature.com/nbt/focus/maqc/>).

► Gene Expression Analysis

QT Prolongation

The QT interval is measured in an electrocardiogram (ECG) between the onset of ventricular depolarisation (QRS) and the end of the repolarization process (T wave). The QT interval is rate-dependent and may be altered by numerous pathophysiologic and pharmacologic influences. The QT interval corrected for heart rate is termed QTc; its prolongation is known to be associated with various malignant tachyarrhythmias and specifically torsades de pointes. Drugs which prolong the cardiac action potential, lead to a prolongation of the QTc interval on the electrocardiogram. This may for instance be the case for drugs, which delay cardiac repolarisation (e.g. class III antiarrhythmic drugs). In addition antibacterial agents, such as macrolides and quinolones, as well as drugs from other classes, have the potential to prolong the QTc interval. A slight prolongation does not represent a risk per se, but when these drugs are combined with other agents known to prolong the QT interval in at-risk patients possible untoward effects might occur.

► Antiarrhythmic Drugs

Quantification of Drug Effect

► Drug–Receptor Interaction

Quantitative PCR

Quantitative polymerase chain reaction, also called real-time RT-PCR or QPCR, is a method which employs insertion of a signal, such as fluorescence or enzyme activity, into PCR products generated by RT-PCR to determine the amount of messenger RNA (mRNA) in a tissue accurately.

► Gene Expression Analysis

Quinolinic Acid

► Kynurenine Pathway

Quinolones

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Definition

The term “quinolone antibacterials” describes a large group of drugs that are 4-quinolone derivatives with a

carboxylic acid moiety in position 3 of the basic ring structure or 1,8-naphthyridone (8-aza-quinolone) derivatives. They are active against a broad spectrum of gram-negative and gram-positive pathogens. They exhibit their antibacterial action by inhibiting topoisomerase II (so-called ►gyrase) and topoisomerase IV, which are enzymes that control structure and function of bacterial DNA. ►Topoisomerases in eukaryotic, mammalian cells are not inhibited by quinolone antibacterial agents. Among the quinolones in clinical use today, four groups can be distinguished: those which inhibit gram-negative bacteria, but are not sufficiently active against pneumococci (groups I and II, e.g. ciprofloxacin), those which exhibit increased activity against a broad spectrum of bacteria, including pneumococci (group III, e.g. levofloxacin) and those which act against a broad spectrum with high anti-pneumococcal activity and are also active against some anaerobic bacteria (group IV, e.g. moxifloxacin and gatifloxacin). All of these compounds are fluorinated in position 6 of their basic heterocyclic structure and therefore are also termed ►“fluoroquinolones”. However, quinolones without 6-fluorination are under development (e.g. garenoxacin, PGE9262932) and thus the term “quinolones” seems to be more appropriate for the whole group of antibacterials. According to their differences in antibacterial activity and also their pharmacokinetic properties the indications differ for their clinical use. When used therapeutically, also their potential toxicities and possible adverse reactions have to be taken into account [1].

Mechanism of Action

The ►chromosomes of *Escherichia coli* and other bacteria are single, double-stranded DNA molecules with a total length of more than 1,000 µm. Relaxed DNA exists as a helical molecule, with one full turn of the helix occurring approximately every 10.4 base pairs. This molecule must undergo several folding and compaction steps to fit into an *E. coli* cell which is only 1–3 µm long. Despite this enormous compaction, bacterial DNA must be accessible for the bacterial enzymes that catalyze DNA replication and transcription

into mRNA, the prerequisite for protein synthesis. These and other topological problems are solved by enzymes called DNA topoisomerases. DNA gyrase (topoisomerase II) was the first quinolone target identified on the basis of genetic studies with *E. coli* mutants that were resistant against nalidixic acid (i.e. the first quinolone antimicrobial which is no longer in clinical use). DNA gyrase has a tetrameric A₂B₂ structure (Table 1); the two subunits of gyrase are encoded by the *gyrA* and *gyrB* genes. The enzyme is responsible for introducing negative ►supercoils into DNA – negatively supercoiled DNA contains slightly less than one helical turn per 10.4 base pairs. This is an ATP-dependent reaction that requires both strands of the DNA be cut to permit passage of a segment of DNA through the break; the cleavage is then resealed. Topoisomerase IV is a homologue of gyrase, which is encoded by the *parC* and *parE* genes. This enzyme catalyzes the unlinking of replicated daughter chromosomes, a process that is called decatenation. Both enzymes cause double strand breakage of DNA strands and are called “type II topoisomerases” in contrast to “type I topoisomerases” that catalyze single-stranded DNA cleavage [2].

Quinolones inhibit bacterial DNA synthesis by their ability to bind to and to stabilize complexes of DNA and type II topoisomerases; there is strong evidence for a role of Mg²⁺ ions in this process. The enzymes break DNA and the quinolones prevent re-ligation of the broken DNA strands. Inhibition of DNA synthesis by interaction with gyrase occurs rapidly, but inhibition due to interaction with topoisomerase IV occurs with some delay. This is thought to relate to differences in the localization of DNA gyrase and topoisomerase IV on the bacterial chromosome; gyrase works ahead of replication forks and topoisomerase IV is located behind replication forks. Differences exist with respect to the primary targets of quinolones in different bacteria. For *E. coli*, DNA gyrase is more sensitive to many quinolones than topoisomerase IV, but for the gram-positive pathogen *Staphylococcus aureus* topoisomerase IV is the more sensitive of the two enzymes. The more sensitive enzyme usually represents the

Quinolones. Table 1 Quinolones – Classification of bacterial topoisomerases

Topoisomerase	Type	Structure	Gene	Predominant function in cell
I	I	1 subunit		Relaxes negatively supercoiled DNA
II (“gyrase”)	II	Tetramer (2 GyrA; 2 GyrB subunits)	<i>gyrA</i> / <i>gyrB</i>	Introduces negative supercoils into DNA
III	I	1 subunit		Decatenation of replication intermediate
IV	II	Tetramer (2 ParC; 2 ParE subunits)	<i>parC</i> / <i>parE</i>	Decatenation of linked daughter DNA molecules

primary quinolone target for a given organism. Events in addition to interaction of the quinolone with its target enzyme DNA complex are necessary for the rapid bactericidal action of quinolones, but these events are poorly understood. Probably at least two different lethal modes of action exist, one that requires protein synthesis and one that does not [3].

Most of our knowledge about the targets of quinolone action is the result of extensive studies performed to understand (and possibly overcome) the phenomenon of bacterial ►resistance against quinolones, which represents an increasing problem with the use of these and other antimicrobial agents. In *E. coli* and other gram-negative bacteria first step quinolone resistance mutations occur in *gyrA*, or less commonly, *gyrB*. In contrast, for many gram-positive bacteria, as for *S. aureus*, first-step mutations occur in *parC*, less commonly *parE*. To avoid the development of double mutants, the quinolone should be active enough to destroy pathogens with reduced susceptibility due to first step resistance. An optimal bactericidal effect requires the AUC/MIC ratio to be greater than 100, selection of resistant mutants is unlikely with a C_{max}/MIC ratio greater than 10.

Interestingly, rather slight modifications of the molecular structure of antibacterial quinolones can render them into substances that have the potential to inhibit mammalian topoisomerases. One of such compounds is CP-115,953 which is a more potent inhibitor for mammalian topoisomerases than etoposide, a drug that is used as a cytostatic agent in cancer patients. CP-115,953 and similar derivatives were not further developed for clinical use [4]. Because any inhibition of mammalian topoisomerases is unwanted with drugs used for the treatment of bacterial infections, quinolones are tested at very early stages during their preclinical development as to whether they exhibit such a potential. However, these antineoplastic quinolones represent a potentially important source of new anticancer agents.

Clinical Use

Besides the four most widely used fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin) several other derivatives of the same basic structure are available for clinical use. However, several shortcomings, such as relatively low antibacterial activity or poor pharmacokinetics, do not render them drugs of first choice. For example, enoxacin has a relatively low antibacterial activity and exhibits a pronounced potential for ►drug interactions by inhibition of CYP1A2 (theophylline metabolism). Another example is ofloxacin that represents a racemate consisting of equal parts of two substances (the R- and L-forms), but only one of these compounds – the L-form, also called levofloxacin – exhibits antibacterial activity. Thus, from a pharmacological point of view levofloxacin is the more rational choice than ofloxacin. The indications for the four most often used quinolones differ due to differences in their antimicrobial spectrum as well as their pharmacokinetics. Table 2 provides an overview of the indications of these drugs as licensed in Germany or other countries. They are available for oral as well as for intravenous application (gatifloxacin is available in the USA, but not in Europe).

As with all drugs, the specific side effects of the quinolones must be considered when they are chosen for treatment of bacterial infections [5]. Reactions of the gastrointestinal tract and the central nervous system are the most often observed adverse effects during therapy with quinolones. It should be underlined, however, that compared with many other antimicrobials, diarrhea is less frequently observed during quinolone treatment. Antibiotic-associated colitis has been observed rarely during quinolone therapy. Similarly, hypersensitivity reactions, as observed during therapy with penicillins and other β -lactams, is less frequently caused by quinolones. Some other risks of quinolone therapy have been defined and must be considered if a drug from this class is chosen for treatment of bacterial infections.

Quinolones. Table 2 Quinolones

Indication	Ciprofloxacin	Levofloxacin	Moxifloxacin	Gatifloxacin
Uncomplicated UTI*	X	X		X
Complicated UTI	X	X		X
Community-acquired pneumonia		X	X	X
Acute exacerbation of chronic bronchitis		X	X	X
Sinusitis		X	X	X
Uncomplicated skin and skin structure infections	X	X	X	X
Pseudomonas infections	X			
Sepsis	X			

*UTI = Urinary tract infection ("uncomplicated" = no obstruction).

Because quinolones can cause prolongation of the QTc-interval, they should not be used (i) in patients with inborn ►QT prolongation, (ii) in patients treated with other drugs that cause QT prolongation (e.g. antiarrhythmics), (iii) in patients with electrolyte disturbances, such as hypokaliemia or hypomagnesemia or (iv) in patients with severe heart disease. Alterations of glucose homeostasis have been observed with quinolone use and strict glucose controls of diabetic patients are recommended, especially if they are treated with sulphonylurea agents; such effects occur more frequently with gatifloxacin than with other quinolones. Damage of articular joint cartilage as well as the epiphyseal growth plate can be induced by quinolones in immature animals and these chondrotoxic effects have led to a restricted use of quinolones in pediatrics. Another manifestation of the connective tissue toxicity of quinolones is tendopathies. ►Tendinitis and tendon ruptures have occurred in rare cases as late as several months after treatment with quinolones.

A number of quinolones had to be taken off the market due to toxic effects on the liver, heart, or other organs, that became recognized only after marketing (e.g. temafloxacin, trovafloxacin, grepafloxacin). A risk for severe cardiotoxicity, hepatotoxicity, or phototoxicity is

not associated with the clinical use of those four quinolones (ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin) described as preferential quinolones in this chapter.

►Microbial Resistance to Drugs

►Ribosomal Protein Synthesis Inhibitors

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RAAS

► Renin–Angiotensin–Aldosterone System

Rab-GTPases

Rab proteins are a family of small GTPases related to ras. Rab proteins possess GTP-binding motifs, an effector loop that changes conformation concomitant with the GTP-GDP cycle and a C-terminal CXC or CC box that serves as attachment for the hydrophobic geranylgeranyl membrane anchors. Each intracellular membrane carries a specific set of Rab proteins. The GTP-GDP cycle of rab proteins is regulated by a complex network of protein-protein interactions. The GDP form of rabs is recognized by a soluble protein termed GDI (for GDP dissociation inhibitor). GDI dissociates GDP-rabs from the membrane and allows for re-binding, thus ensuring that rabs are not carried along the secretory pathway, which would result in a loss of membrane specificity.

Membrane-bound GTP rabs recruit effectors to the membrane. In neurons and neuroendocrine cells, the vesicle-associated Rab3 binds to rabphilin and to RIM. RIM is a component of the presynaptic cytomatrix and may thus serve as a docking receptor for synaptic vesicles at the active zone.

- Exocytosis
- Intracellular Transport
- Small GTPases

Radiocontrast Agents

► X-ray Contrast Agents and Molecular Imaging

Radioiodine

The ^{131}I isotope of iodine is used for treatment of thyroid disorders. ^{131}I -iodine is administered orally as a single dose. It is rapidly and completely absorbed and is accumulated in the thyroid gland via an active transport mechanism. Thereafter it is oxidized and organified by thyroid follicular cells. Its physiologic half-life is 8 days. ^{131}I emits both beta and gamma radiation. The toxic effect on thyroid tissue is caused by the released beta particles, which have a path length of 1–2 mm. They destroy thyroid follicular cells. The additional fraction of gamma radiation is utilized for detection. When used for treatment of benign thyroid disorders (e.g. Graves' disease or thyroid autonomy), exposure of other organs is small. Infrequent side effects of radioiodine therapy are nausea, vomiting and anorexia. Although there is no established teratogenic risk, the use of radioiodine is contraindicated in pregnancy and breast-feeding women. It should be administered within 10 days of the onset of a menstrual period or after a negative pregnancy test and pregnancy should be avoided for 4 months after radioiodine treatment.

► Antithyroid Drugs

Radiomimetics

Radiomimetics are a class of agents that cause DNA damage of a similar quality to that resulting from exposure to ionizing radiation.

► Cancer (Molecular Mechanisms of Therapy)

Raf

Raf kinases such as Raf1 are serine/threonine-specific protein kinases which function in signal transduction

pathways between the cell surface and the nucleus. It is a downstream target of the monomeric GTPase Ras.

► Phospholipid Kinases

RAGE

► Receptor for Advanced Glycation End Products

Rapamycin Analogs

Rapalogs have been designed to be used in several different applications. Rapalogs used clinically have been designed to have superior pharmacokinetic properties. Other rapalogs have been designed to be used as experimental reagents. For example, some rapalogs induce heterodimerization of FRB- and FKBP-domain-containing protein construct but have lost the ability to inhibit TOR/mTOR activity.

► TOR Signalling

Raphe Nuclei

The raphe nuclei are a cluster of nuclei found in the brainstem, where they are located in the medial portion of the formatio reticularis, the raphe. (The raphe is the junction of the left and right brainstem hemisphere, hence the name raphe=seam). Serotonergic nerve cells in the CNS originate from the raphe nuclei, i.e., their rostral portion, and because of their wide-ranging projections appear to supply serotonin (5HT) to the rest of the brain.

► Neurotransmitter Transporters
► Serotonergic System

Rapid Eye Movement Sleep

Synonyms

REM Sleep

Definition

REM sleep is a paradoxical *sleep stage*, in that the brain activity as measured by the EEG resembles that of wake. Specifically, the EEG signal consists of high frequency, low amplitude activity, with a prevalent “theta” (4–8 Hz) component. In contrast to wake, however, during REM sleep, muscle atonia occurs, thereby inhibiting movement. REM sleep is so named because specific patterns of brain activity intrinsic to this sleep stage result in rapid movements of the eyeballs, as detected by *electro-oculogram* electrodes. It is though that dreaming occurs primarily during REM sleep, based on reports of subjects waking at various points during the sleep–wake cycle, although some dreaming activity was found to occur during non-REM sleep. Loss of the so-called “REM atonia” can result in the sufferer effectively “acting out their dreams”, as observed in the clinical condition *REM behaviour disorder*.

► Sleep

Ras Protein

Ras is a G protein that cycle between two conformations, an activated Ras-GTP or inactivated form Ras-GDP. Ras, attached to the cell membrane by lipidation, is a key component in many signalling cascades, which couple growth factor receptors to downstream effectors that control such processes as cytoskeletal integrity, proliferation, cell adhesion, apoptosis and cell migration. Mutations and dysregulations of the Ras protein leading to increased invasion and metastasis, and decreased apoptosis are very common in cancers.

Ras activates a number of pathways among them is the mitogen-activated protein (MAP) kinases, which transmit signals downstream to other protein kinases and gene regulatory proteins

► Phospholipids
► Small GTPases

Raynaud's Phenomenon

Raynaud's phenomenon is an exaggerated vascular response to cold temperature or emotional stress. Clinical symptoms are sharply demarcated color changes in the skin of the digits. The underlying disorder consists of abnormal vasoconstriction of digital arteries and cutaneous arterioles due to a local defect in normal vascular responses.

► Ca^{2+} Channel Blockers

often more severe than before the treatment commenced. This often occurs following treatment with short half-life benzodiazepines, particularly if the drug is not gradually reduced but suddenly withdrawn.

► Sleep

Rb Gene

► Retinoblastoma (Rb) Gene

Receptor for Advanced Glycation End Products

Receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin-like cell surface receptor superfamily inter-acting with several S100 proteins.

► S100 Proteins

Reactive Oxygen Species

Synonyms

► ROS

Definition

Reactive oxygen species like superoxide, hydrogen peroxide (H_2O_2) or hydroxyl radicals (OH) are formed in the course of a variety of enzymatic processes in cells. Although potentially very toxic, their concentrations are kept low due to the activity of various antioxidants. However, under pathological conditions reactive oxygen species can accumulate resulting in oxidative stress which may lead to cellular damage due to the oxidation of NA, proteins, or lipids.

► Antioxidants

Receptor Occupancy

Receptor occupancy refers to the ratio of receptors occupied by a ligand at equilibrium and the total number of receptors available, usually expressed as a percentage of the total number of receptors. Since it is often not possible to quantify the total number of receptors or the number of receptors occupied by a ligand, another parameter called binding potential is often used to measure receptor occupancy. Binding potential (BP) refers to the ratio of the maximum number of receptors and the equilibrium dissociation constant of the drug, so that % receptor occupancy equals to

$$100 \times \text{BP}_{\text{baseline}} - \text{BP}_{\text{post-treatment}} / \text{BP}_{\text{baseline}}.$$

► Drug–Receptor Interaction

Rebound Insomnia

A condition following treatment of *insomnia*, when on cessation of medication, the insomnia reoccurs and is

Receptor Protein

► Table appendix: Receptor Proteins

Receptor Reserve

In terms of the relationship between receptor occupancy by an agonist and tissue response, if the maximal response to the agonist can be obtained with concentrations of agonist that do not occupy all of the receptors, then the system is said to have a receptor reserve. For example, if only 10% of the receptors need be occupied by a certain agonist to produce the system maximal response, then there is a 90% receptor reserve. Under these circumstances, 90% of the receptors could be irreversibly removed (i.e., by an irreversible antagonist) and the agonist would still produce the maximal response. However, the dose-response curve to the agonist would be shifted to the right by the presence of the irreversible antagonist. It should be noted that receptor reserve is not a property of the system but rather that it varies with different agonist efficacies in different systems.

► Drug–Receptor Interaction

Receptor Serine/Threonine Kinases

Receptor serine/threonine kinases function as hetero-oligomeric complexes, consisting of type I and type II serine/threonine kinase receptors. Ligand binding induces the oligomerization. Type II-receptors primarily bind the ligand and phosphorylate type I-receptors, which then specifically phosphorylate in turn receptor-regulated Smad-proteins (R-Smads). These then dimerize with Co-Smads in the cytosol. The R-Smad/Co-Smad complex then translocates to the nucleus where it binds to regulatory sequences in combination with specific transcription factors.

- Neurotrophic Factors
- SMADs
- Table appendix: Receptor Proteins

Receptor Subtype

Most hormones and neurotransmitters can interact with more than one receptor subtype. The different receptor isoforms may differ in their ligand-binding properties,

tissue distribution and signal transduction patterns. Subtype-specific ligands (agonists or antagonists) allow cell type- or tissue-specific targeting of receptor subtypes and thereby increase the specificity of cellular responses.

► Transmembrane Signaling

Receptor Tyrosine Kinases

A subclass of tyrosine kinases functioning as receptors for a variety of regulatory molecules, including growth factors and hormones. Ligand binding usually results in receptor dimerization and tyrosine autophosphorylation, which in turns initiates a signal transduction cascade. Mutations in receptor tyrosine kinases have been implicated in various malignancies.

- Vascular Endothelial Growth Factor
- Insulin Receptor
- Table Appendix: Receptor Proteins
- Growth Factors

Receptors

Scientists in the nineteenth century realized that some chemicals produced effects at extremely low concentrations and that these effects could be modified by equally low concentrations of other chemicals with a strict adherence to chemical structural guidelines. Thus, they proposed that “receptors” with strict recognition properties for these chemicals existed in biological systems. While receptors technically can be any biological entity such as enzymes, reuptake recognition sites, and genetic material such as DNA, the term is usually associated with proteins on the cell surface that transmit information from chemicals to cells. The most therapeutically relevant receptor class is G-Protein-Coupled Receptors (GPCR’s) presently comprising 45% of existing therapies.

- Drug Interactions
- Drug–Receptor Interaction
- G-protein-coupled Receptors
- Tolerance and Desensitization
- Transmembrane Signaling

Recombinant Protein

Recombinant protein is a protein produced by a genetically modified cell or an organism, which expresses a DNA that was subjected to recombination and inserted into its genome.

Rectification

In many channels the relation between current and membrane potential is nonlinear. These channels show rectification, i.e., the property of the channel to conduct ions more readily in one direction than in the other. Depending on the preferential current flow through channels, these are described as outward or inward rectifying.

- ▶ Inward Rectifying K^+ Channels
- ▶ Ionotropic Glutamate Receptors

5 α -Reductase

5 α -Reductase is an enzyme, which converts testosterone to dihydrotestosterone, which has a greater affinity for androgen receptors. 5 α -Reductase is expressed in various peripheral organs. Inhibitors of 5 α -reductase (e.g., finasteride) are used to treat benign prostatic hyperplasia.

- ▶ Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

Reelin

Reelin is an extracellular matrix protein, which is secreted by neuronal cells and binds to two lipoprotein receptors (VLDLR and ApoER2) that relay the Reelin signal inside target neurons by docking the tyrosine kinase adapter disabled-1 (Dab1). This allows neurons to complete migration and adopt their ultimate positions in laminar structures in the central nervous system. In

addition, the integrins α -3/ β -1 and protocadherins of the CNR family may also modulate the Reelin signal.

- ▶ Cadherins/Catenins
- ▶ Low Density Lipoprotein Receptor Gene Family

Reentrant Arrhythmia

Reentrant arrhythmia occurs when due to inhomogeneous repolarization or unidirectional block, heart tissue which is no longer refractory is close beside tissue which is still activated. This may result in a circuit propagation of activation serving as a reverberator.

- ▶ Antiarrhythmic Drugs

Regulated Secretion

- ▶ Exocytosis

Regulation of Gene Expression

- ▶ Transcriptional Regulation

Regulation of Ingestive Behaviour

- ▶ Appetite Control

Regulation of mRNA Synthesis

- ▶ Transcriptional Regulation

Regulation of Smooth Muscle Contractility

- ▶ Smooth Muscle Tone Regulation

Regulators of G-protein Signalling

Regulators of G-protein signalling (RGS) are a group of G-proteins which are able to act as GTP-activating proteins (GAPs) for heterotrimeric G-proteins. They are thus able to limit the signal generated by G-protein coupled receptors (GPCRs). RGS-proteins are a large family of structurally diverse proteins, which in addition to an RGS-domain carry variable sequence motifs, which are responsible for additional specific interactions.

- ▶ G-proteins
- ▶ Transmembrane Signalling

- ▶ Leukotrienes
- ▶ NF- κ B
- ▶ Nuclear Factor- κ B
- ▶ Glucocorticoids

Regulatory Myosin Light Chain II

Synonyms

rMLC

Definition

Smooth muscle myosin contains two myosin light chains. Phosphorylation of the regulatory light chain by myosin light chain kinase is a mandatory step to induce contraction.

- ▶ Smooth Muscle Tone Regulation

Regulatory Regions

Regulatory regions are transcriptional control sequences, which consist of promoters, response elements, enhancers and possibly silencers, located upstream of the start site of transcription. The overall effect on gene transcription is a sum of the contributions of these elements and the activities of proteins recruited to these sites. Promoters are located immediately upstream of the start site and initiate transcription. They often contain tissue- or cell-specific elements if the gene is not ubiquitously expressed. Enhancers are positive regulatory elements which function independently of orientation and distance from the genes they regulate.

Regulatory T-cells

Regulatory T cells are a subpopulation of T cells that are morphologically defined by expression of the surface markers CD4 and CD25 and the transcription factor FoxP3. There is growing evidence that regulatory T cells are substantially involved in mediating immunotolerance. This effect could be achieved by direct cell contact with other immune cells and by liberation of certain messenger molecules (cytokines like interleukin-10 or transforming growth factor β).

- ▶ T Cell Receptors

Reinstatement

The reinstatement model is the first choice for the measurement of craving and relapse behavior. In this paradigm, animals are trained to self-administer a drug and are then subjected to extinction – that is, they are tested under conditions of nonreinforcement until operant responding appears to be extinguished. When the animals reach some criterion of unresponsiveness, various stimuli are presented. A stimulus is said to reinstate drug-seeking behavior if it causes renewed responding, i.e., lever pressing, without any further response-contingent drug reward. At least three conditions can reinstate responding: drug priming – that is the injection of a small dose of the drug, stress, and conditioned stimuli.

- ▶ Drug Addiction/Dependence

Rel Homology Domain

The Rel homology domain (RHD) is an evolutionarily conserved domain found in some eukaryotic transcription factors, including NF- κ B, the nuclear factors of activated T-cells (NFATs) and the drosophila proteins Dif and Relish. Some of these transcription factors form

multiprotein DNA-bound complexes. Phosphorylation of the RHD appears to play a role in the regulation of the activity of some of these transcription factors and modulation of expression of their target genes. Structurally, the RHD is composed of two immunoglobulin-like RHD contains the site for interaction with the IκBs.

►Nuclear Factor Kappa B

Rel/NF-κB

Family of transcription factors that modulate the expression of genes which control immune, inflammatory, and acute-phase responses, as well as cell growth, responses to stress, apoptosis, and oncogenesis. All members of this family have a Rel-homology domain that contains sequences responsible for dimerization and DNA binding. In vertebrates, this family includes NF-κB1 (also known as p50), NF-κB2 (also known as p52), Rel (also known as cRel), Rel-A (also known as p65), and Rel-B.

►NFAT Family of Transcription Factors

Rel Proteins

►Nuclear Factor Kappa B

Relative Potency

The affinity of a drug is the ability of that drug to bind to the receptor. The efficacy of an agonist is its ability to activate (or inactivate in the case of an ►[inverse agonist](#)) the receptor. The relative potency of an agonist (with respect to another agonist at the same receptor) is therefore determined by both its affinity and efficacy and only gives an indication of the relative concentrations at which they produce a particular response.

Relaxin

Relaxin and its relative relaxin-like factor (RLF/INSL3) belong structurally to a group of peptide hormones, that includes insulin and insulin-like growth factor 1 (IGF1). Relaxin regulates the growth and remodeling of reproductive tissues during late pregnancy. It promotes expansion of the birth canal during parturition. In humans, the peak in circulating relaxin occurs during the first trimester. Relaxin is secreted by the ovary during pregnancy as well as by many other tissues. It may act as a vasoactive hormone, which has dilatory effects on blood vessels. The closely related relaxin-like factor is synthesized by Leydig cells in the fetal testis and appears to be responsible for the second phase of testicular descent by influencing the growth and differentiation of the cords, which connect the testes with the lower abdomen. The effects of relaxin and relaxin-like factors are mediated at least in part by G-protein coupled receptors (LGR7 and LGR8, which are coupled to G_s).

REM Sleep

Rapid eye movement sleep. Sleep stage characterized by rapid movements of the eyes and asynchronous EEG activity in the theta-frequency (5–10Hz) range. Counterpart is slow wave sleep, characterized by other electrophysiological (synchronized low frequency 1–2Hz, large amplitude EEG and neuronal sharp wave-ripple oscillations) and endocrine (growth hormone surge) activities.

Renal Glucosuria

Renal glucosuria is caused by a low renal threshold for glucose. The clinical picture is the daily loss of 50–60 g of glucose in the urine despite a normal glucose tolerance test. Autosomal dominant and autosomal recessive inheritance has been described. It has been suggested that the disease is caused by mutations in the SGLT2 (sodium-dependent glucose cotransporter 2) gene.

►Glucose Transporters

Renin–Angiotensin–Aldosterone System

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Synonyms

RAAS; Renin–angiotensin system (RAS)

Definition

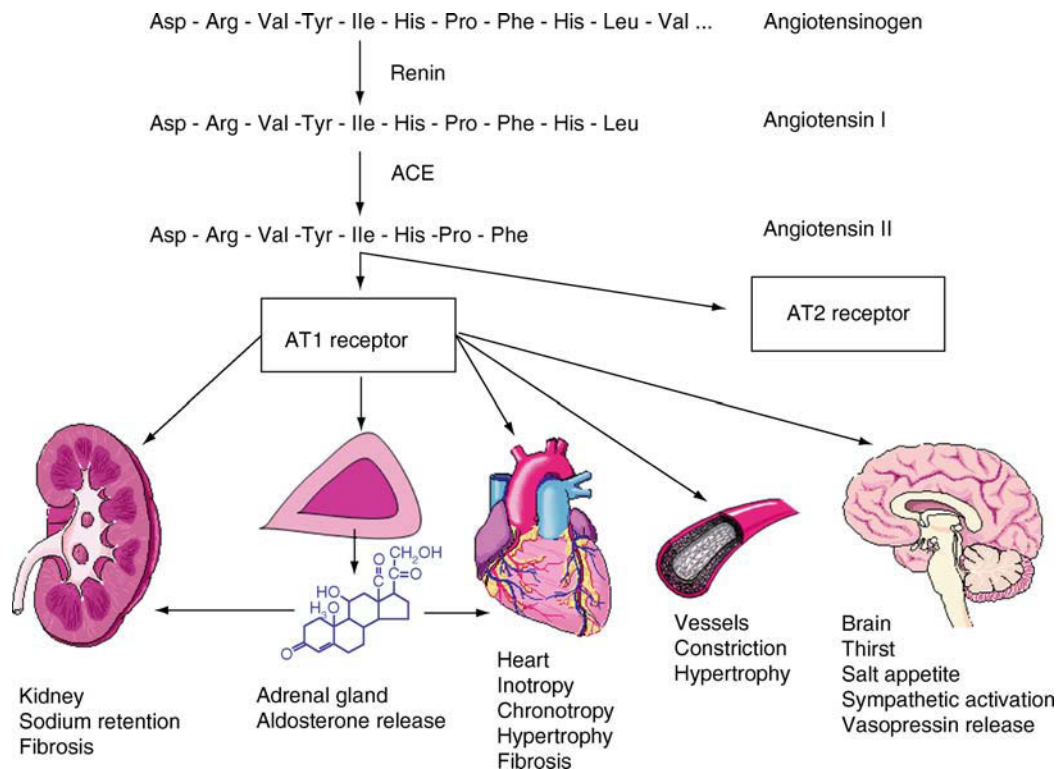
The renin–angiotensin–aldosterone system (RAAS) generates the peptide hormone angiotensin II and subsequently the mineralocorticoid aldosterone, which both exert considerable impact on blood pressure (►blood pressure control) and fluid homeostasis, and

have prime etiologic and therapeutic significance for cardiovascular diseases.

Basic Characteristics

General

Renin is a circulating enzyme formed in specialized smooth muscle cells in the kidney that cleaves its only, liver-born substrate, angiotensinogen, in the plasma to form the inactive decapeptide angiotensin I. Angiotensin I is then metabolized further into the octapeptide angiotensin II via the endothelium-bound angiotensin converting enzyme (ACE). Angiotensin II elicits an increase in blood volume and blood pressure by stimulating vasoconstriction, sodium retention, thirst, the sympathetic nervous system, and aldosterone secretion from the adrenal gland (Fig. 1). Aldosterone is a steroid hormone that binds to the mineralocorticoid receptor and amplifies the sodium-retaining effect. Physiologically this makes sense, since the RAAS is activated under conditions of acute volume loss by the



Renin–Angiotensin–Aldosterone System. Figure 1 The renin–angiotensin–aldosterone system. Angiotensin II is generated in a two-step enzymatic process from the liver-born protein angiotensinogen by the kidney-derived enzyme renin and the endothelium-bound angiotensin converting enzyme (ACE). The octapeptide interacts with two receptors, AT1 and AT2. The AT1 receptor confers most of the known actions of angiotensin II such as the liberation of aldosterone from the adrenal gland. Aldosterone via the mineralocorticoid receptor (MR) and angiotensin II together induce sodium retention in the kidney and fibrotic processes in kidney and heart. Moreover, angiotensin II elicits constriction of vessels, has positive inotropic and chronotropic actions on the heart, promotes growth in vessels and heart, and induces thirst, salt appetite, vasopressin release, and the activation of the sympathetic nervous system in the brain. Some of these effects are also mediated by locally produced angiotensin II in the respective tissues.

induction of the rate-limiting enzyme renin. Major renin-inducing stimuli include a fall in renal perfusion pressure, a decrease in salt content in the distal tubule sensed and transmitted via the ►**macula densa**, an increase in the renal sympathetic tone, and a reduction in angiotensin II concentration employing a negative feedback mechanism. In addition to their hemodynamic actions, angiotensin II and aldosterone also induce growth- and fibrosis-related processes in several organs, such as vessels, heart, and kidney. Recently, a receptor for renin has been discovered, which activates the enzyme and exerts angiotensin-II dependent and independent signaling effects, which are deleterious for the heart and the kidney [1].

To elicit these effects, angiotensin II binds to two main receptors, AT1 and AT2, which both belong to the ►**G-protein-coupled receptor** family. Most of the abovementioned effects of angiotensin II are, however, mediated by the AT1 receptor, while the physiological function of the AT2 receptor is enigmatic. In most studied cases, the AT2 receptor counteracts the AT1 effects by exerting growth-inhibiting and vasodilating actions partly by the stimulation of kinin generation.

Angiotensin II binding to the AT1 receptor stimulates via the G-protein, G_q , the activity of phospholipase C to generate the second messengers inositol phosphate (IP3) and diacylglycerol, and inhibits via G_i the activity of adenylyl cyclase to reduce the synthesis of cyclic AMP. Diacylglycerol activates protein kinase C and can be converted to arachidonic acid and ►**eicosanoids**. Furthermore, angiotensin II induces the generation of ►**reactive oxygen species** by the stimulation of membrane-bound NAD(P)H oxidase. One of the immediate consequences of these early signals is activation of tyrosine kinases that include PYK2, c-Src, JAK2, platelet-derived growth factor (PDGF) receptor and the epidermal growth factor (EGF) receptor, as well as of the serine/threonine kinases, ERK, Akt/protein kinase B and S6 kinase, and subsequent induction of ►**immediate early genes** and protein synthesis.

Besides the plasma renin–angiotensin system (RAS), intrinsic tissue RAS exist [2]. Angiotensin II is generated not only in the circulation but also locally in organs from precursors and enzymes either locally synthesized or imported from the plasma. These systems are autonomously regulated and have physiological functions inside the respective organs. Local RAS have been described for organs involved in cardiovascular control such as kidney, vessels, heart, adrenal gland, and brain.

Adrenal Gland

In the ►**zona glomerulosa** of the adrenal gland, renin is locally synthesized, and together with angiotensinogen and ACE generates angiotensin II, which acts as

paracrine or intracrine hormone on adrenocortical cells. Circulating as well as this locally produced angiotensin II stimulates aldosterone release by increasing the expression of aldosterone synthase, the rate-limiting enzyme of aldosterone synthesis.

Kidney

The kidney contains the major site of renin synthesis, the juxtaglomerular cells in the wall of the afferent arteriole. From these cells, renin is secreted not only into the circulation but also into the renal interstitium. Moreover, the enzyme is produced albeit in low amounts by proximal tubular cells. These cells also synthesize angiotensinogen and ACE. The RAS proteins interact in the renal interstitium and in the proximal tubular lumen to synthesize angiotensin II. In the proximal tubule, angiotensin II activates the sodium/hydrogen exchanger (NHE) that increases sodium reabsorption. Aldosterone elicits the same effect in the distal tubule by activating epithelial sodium channels (ENaC) and the sodium–potassium-ATPase. Thereby, it also induces water reabsorption and potassium secretion.

In the interstitium, angiotensin II induces proliferation of mesangial cells and fibroblasts and the synthesis of collagen and other matrix molecules by these cells via the AT1 receptor. Moreover, by the concomitant stimulation of chemoattractant ►**cytokines**, inflammation is induced. These processes are mediated by endothelin, ►**transforming growth factor** β , and reactive oxygen species, and finally lead to interstitial fibrosis and glomerulosclerosis observed in hypertension and diabetes.

Heart and Vessels

In heart and vessels, angiotensin II is generated mostly by renin and angiotensinogen imported from the plasma and locally synthesized ACE. Additionally in the human heart, mast cells contain the enzyme chymase that also metabolizes angiotensin I to angiotensin II. The physiological relevance of this enzyme is controversial. Circulating as well as locally generated angiotensin II induces vasoconstriction and exerts direct inotropic and chronotropic actions on the heart. These effects are enhanced by a facilitation of noradrenaline release from sympathetic nerve endings.

Alike in the kidney, angiotensin II also in the heart induces inflammation and fibrosis by increasing endothelin, transforming growth factor β , reactive oxygen species, and proinflammatory cytokines. Furthermore, angiotensin II induces hypertrophy of cardiomyocytes and smooth muscle cells in the heart and vessels, respectively, partially employing the same mediators. This is aggravated by increased circulating aldosterone levels that also elicit fibrotic processes in the heart by a yet unresolved mechanism.

Brain

Circulating angiotensin II can only reach the ►**circum-ventricular organs** of the brain, which express AT1 receptors and lack a blood–brain barrier normally limiting the access of peptides to brain receptor sites. However, areas beyond the blood–brain barrier have been shown to be responsive to angiotensin II and to express AT1 receptors. These sites are affected by locally synthesized peptide from renin, angiotensinogen, and ACE present in the central nervous system. Possibly, also other enzymes may be involved in angiotensin II generation in the brain, e.g., cathepsins. Circulating and locally synthesized angiotensin II induces thirst, salt appetite, and ►**vasopressin** release, stimulates the sympathetic nervous system and moderates the ►**baroreceptor reflex**, and thereby increases blood volume and blood pressure.

Drugs

ACE Inhibitors

Pharmacological intervention in RAS began in the late 1960s with the discovery that the venom of the Brazilian snake *Bothrops jararaca* contains a substance that inhibits ACE. In first clinical trials this substance proved to be a potent antihypertensive agent but it had the disadvantage that it could only be taken by injection. By modeling the active site of ACE and designing drugs potentially binding to this site, the first orally available ACE inhibitor, captopril, was discovered. In the meantime, at least a dozen pills have been developed and marketed: captopril, enalapril, lisinopril, perindopril, cilazapril, benazepril, quinapril, fosinopril, ramipril, moexipril, and trandolapril. ACE inhibitors are first choice antihypertensive drugs. Furthermore, a multitude of large-scale clinical studies have proven a strong beneficial effect of these drugs on morbidity and mortality in congestive heart failure, e.g., after myocardial infarction, and chronic renal diseases, e.g., caused by diabetes or hypertension. In the heart, ACE inhibitors exert their beneficial actions by reducing preload and afterload and inhibiting myocardial fibrosis and remodeling processes. In the kidney, a reduction in glomerular pressure as well as antifibrotic and antiinflammatory actions contribute to the efficiency of the drugs.

ACE not only activates angiotensin but is also involved in the metabolism of other peptides, e.g., it is a major kinin-degrading enzyme. Therefore, ACE inhibitors also increase kinin concentrations. Furthermore, it has recently been shown that these drugs potentiate kinin effects by modulating a direct interaction between the ACE protein and the kinin B2 receptor, which is independent from the enzymatic activity of ACE. Kinin potentiation may be involved in the beneficial action of ACE inhibition since ►**kinins** are known to exert cardio- and renoprotective actions.

However, it is also the major reason for the adverse side effects of ACE inhibitors, namely cough and angioedema. Another observed side effect, first-dose orthostatic hypotension, is probably due to both angiotensin inhibition and kinin potentiation.

Since angiotensin II is important for kidney development in mammals, ACE inhibitors and other drugs interfering with RAS should not be given during pregnancy.

AT1 Antagonists

A second group of drugs interfering with RAS are specific antagonists for the AT1 receptor. The first example of this class was losartan which was followed by at least six other sartans (telmisartan, candesartan, valsartan, eprosartan, irbesartan, olmesartan). These drugs exert a more complete angiotensin blockade, since alternative pathways of angiotensin generation not affected by ACE inhibitors and employing cathepsins or chymase become ineffective by AT1 antagonism. They are also more specific for the RAS than ACE inhibitors, since other peptide systems should not be affected. However, the compensatory increase in renin concentration after AT1 blockade leads to an accumulation of angiotensin II, which activates the AT2 receptor. It is yet unknown whether this AT2 stimulation often followed by kinin generation is involved in the action of AT1 antagonists.

In clinical trials, AT1 antagonists have proven to be as effective as ACE inhibitors in hypertension, congestive heart failure, and renal failure [3]. The favorable side effect profile of AT1 antagonists argues for a greater use of these drugs. At present, due to still higher costs, they are indicated in patients who do not tolerate ACE inhibitor treatment.

Renin Inhibitors

The most logical point to interfere pharmacologically with the RAS is the rate-limiting enzyme renin. Intervention at this step is more specific than ACE inhibition and AT1 antagonism, since hardly any angiotensin peptide can be generated and no other peptide system is directly affected. Therefore, fewer compensatory and unwanted effects are expected, and thus renin inhibitors should be safe drugs. However, the development of such drugs faced several problems: First, since the human renin protein is different from the rodent enzymes and only interacts with primate or human angiotensinogen, the testing of such drugs can not be performed in classical animal models. Second, the first renin inhibitors were difficult to synthesize and exhibited a low bioavailability. However, with the availability of a transgenic rat model expressing the human renin and angiotensinogen genes as test system novel substances could be developed. Now,

a first renin inhibitor, aliskiren, has been approved for clinical use after first clinical trials which have proven its safety and efficacy [4]. Future large trials will be necessary to compare renin inhibitors with ACE inhibitors and AT1 antagonists, and to evaluate whether these substances also interfere with the renin/renin receptor interaction and thereby elicit additional beneficial effects.

Vasopeptidase Inhibitors

(►Vasopeptidase inhibitors) A new class of drugs has been developed that inhibits ACE and neutral endopeptidase 24.11 (NEP). NEP degrades vasodilatory peptides such as kinins, ►natriuretic peptides, and adrenomedullin, and, therefore, its inhibition should complement the vasodilatory action of ACE inhibition. Since ACE and NEP are very similar in structure it was possible to develop inhibitors with dual specificity for both enzymes such as omapatrilat, sampatrilat, and gemopatrilat. In first clinical trials, the patrilats have proven to be even more effective than ACE inhibitors in blood pressure reduction and in improving congestive heart failure, but more side effects have been observed, probably due to the fact that NEP metabolizes a multitude of peptide substrates. Thus, the usefulness of this class of drugs has been questioned.

Aldosterone Antagonists

ACE inhibitors do not completely block aldosterone synthesis. Since this steroid hormone is a potent inducer of fibrosis in the heart, specific antagonists, such as spironolactone and eplerenone, have recently been very successfully used in clinical trials in addition to ACE inhibitors to treat congestive heart failure [5]. Formerly, these drugs have only been applied as potassium-saving diuretics in oedematous diseases, hypertension, and hypokalemia as well as in primary hyperaldosteronism. Possible side effects of aldosterone antagonists include hyperkalemia and, in case of spironolactone, which is less specific for the mineralocorticoid receptor than eplerenone, also antiandrogenic and progestational actions.

- ACE Inhibitors
- Blood Pressure Control
- Glucocorticoid Receptors

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Repolarization

Repolarization is a return of membrane potential to its resting value. It refers mostly to repolarization of an action potential, although a more general meaning of returning a membrane potential back to a more negative value after (forced) depolarization is also common.

- Antiarrhythmic Drugs

Resiniferatoxin

Resiniferatoxin (RTX) is the toxin isolated from the spurge *Euphorbia resinifera* which is responsible for the powerful burning sensation and skin irritation induced by the milky sap of these plants. Like capsaicin, resiniferatoxin activates TRPV1 currents, but not the currents through other members of the TRPV subfamily.

- TRP Channels
- Vanilloid Receptor

Resistance Plasmid

Extrachromosomal autonomously replicating circular closed DNA molecules encoding non-essential supplementary genetic information, like antibiotic resistance or metabolic capacities.

- Bacterial Resistance to Antibiotics

Respiratory Burst

The respiratory burst is the ability of phagocytes to destroy pathogens by the release of a variety of toxic products including, hydrogen peroxide, superoxide anion and nitric oxide. Production of these toxic metabolites is induced by the binding of aggregated antibodies to the Fc gamma receptors.

Restenosis

Restenosis is the phenomenon of vascular reocclusion postangioplasty or stent.

Resting Potential

Resting potential is a stable membrane potential in nonexcitable cells, or the most stable membrane potential between Action Potentials in excitable cells. In some excitable tissues it is impossible to define a resting potential because of continuous change in membrane potential.

► Inwardly Rectifying K^+ Channels

Resting Tremor

Resting tremor is a tremor present at rest, which usually abates during voluntary movements. Its frequency in Parkinsonism is 4–8 Hz and it occurs most often in the distal extremities.

► Parkinson's Disease
► Anti-Parkinson Drugs

Restless Leg Syndrome

Restless legs syndrome, also known as Ekbom's syndrome, causes unpleasant sensations in the legs such as tingling, discomfort and sometimes pain. Symptoms are usually worse at night and disturb sleep. Resting usually makes symptoms worse and activity relieves the condition. The cause of the condition is currently unknown but it is common and usually occurs in middle age. There are currently few treatments in place.

► Sleep

Retinal

Vitamin A.

► Retinoids

Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a common inherited retinopathy associated with progressive retinal atrophy, and deposition of pigment. The final stage of RP are severe visual impairment or blindness. The genetic and clinical heterogeneity of RP is remarkably high. In the majority of the cases, however, mutations are found in the gene for the light receptor rhodopsin.

► Protein Trafficking and Quality Control

Retinoblastoma (Rb) Gene

The retinoblastoma protein was identified originally through studies of an inherited form of eye cancer in children, known as retinoblastoma. The loss of both copies of the Rb gene leads to excessive cell proliferation in the mature retina. This suggested that

Rb is the product of a tumour suppressor gene. Later it was shown that the retinoblastoma protein is a key regulator of a gene regulatory protein called E2F which plays a key role in the regulation of the cell cycle. E2F function is controlled primarily by the Rb protein. Rb binds to E2F and blocks the transcription of S-phase genes. When cells are stimulated to divide by extracellular signals, active G₁-Cdk accumulates and phosphorylates Rb, reducing its affinity for E2F. The Rb protein then dissociates, allowing E2F to activate S-phase gene expression.

► Cell Cycle Control

Retinoic Acid

Retinoic acid (RA) describes a group of vitamin A acid (synonym Vitamin A1 acid) derivatives such as all-*trans*-retinoic acid (tretinoin), 9-*cis*-retinoic acid and 13-*cis* retinoic acid (isotretinoin). Retinoic acids act through binding to retinoic acid and retinoid X response elements.

► Retinoic Acid Receptors

► Retinoids

Retinoic Acid Receptors

Retinoic Acid receptors (RARs) are nuclear receptors. Nuclear receptors are a large family of structurally related ligand-inducible transcription factors, including steroid receptors (SRs), thyroid/retinoids receptors (TR, RARs and RXRs), vitamin D receptors (VDR), LXR, peroxisome proliferator activated receptors (PPARs), estrogen receptors (ERa and ERb), and orphan receptors for which no ligand has been yet identified. While having in common a modular structure, they are activated by distinct lipophilic small molecules such as glucocorticoids, progesterone, estrogens, retinoids, and fatty acid derivatives.

All nuclear receptors have a hydrophobic pocket into which its specific ligand binds, with helix 12 (H12) being the key response element of NR's. When an agonist is bound to a NR, H12 is oriented antiparallel to H11, capping the ligand binding pocket. This leaves a hydrophobic groove exposed for the binding of coregulator proteins. When an antagonist is bound, H12 is displaced via an extended side chain. H12 moves outward, rotates, and packs into the hydrophobic

groove between helices 3, 4, and 5. As a result, coactivators needed for transcription cannot bind.

Several loci encoding RAR isoforms have been identified in mammals, RAR- α , - β and - γ . They respond to at-RA, 9-*cis*-RA and 13-*cis*-RA. The RARs show spatially restricted distribution patterns during embryogenesis, which have led to speculation on a variety of roles for RA in developmental processes. As with other enhancer-binding proteins, nuclear receptors act as transcription factors by binding to specific DNA recognition sequences generally located upstream of responsive genes. Although RARs can activate gene expression through binding to thyroid hormone response elements, much more specific and potent RA response elements (RAREs) have been identified within the promoter of the RAR gene. These RAREs are essential for RA induction of the RAR-gene and, when linked to heterologous promoters, can confer transcriptional activation via all three RARs.

► Retinoids

Retinoid-resistance

The major limitation to the application of retinoids is the retinoid resistance observed in cancer cells. This means that such cells do not respond either to physiological or therapeutic concentrations of retinoids. Silencing of RAR β has been suggested to contribute to the tumorigenicity and retinoid resistance of lung and breast cancer cells. RAR β silencing is caused by epigenetic changes, namely DNA methylation and repressive chromatin remodeling of the RAR β P2 promoter. Epigenetic changes can be reversed. Pili et al. developed a strategy to overcome the silencing at RAR β by specifically targeting the epigenetic defect at RAR β P2, using combinations of histone deacetylase inhibitors (HDACIs), a class of chromatin remodeling drugs and RAR β agonists.

► Retinoids

Retinoid X Receptor

The retinoid X receptor (RXR) is a nuclear receptor that binds and is activated by certain endogenous retinoids, such as 9-*cis*-retinoic acid. RXR is the obligatory heterodimerization partner for a large number of nonclassic steroid nuclear receptors, such as thyroid hormone receptor, vitamin D3 receptor, peroxisome proliferator-activated receptor and pregnane X receptor.

- Retinoids
- Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes
- Nuclear Receptors

Retinoids

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Synonyms

Retinoids; Vitamin A; Carotinoids; Retinylesters; Retinol; Retinal; Retinoic acid; Vitamin A acid

Definition

Vitamin A (retinol) and its naturally occurring and synthetic derivatives, collectively referred to as retinoids (chemical structure), exert a wide variety of profound effects in apoptosis, embryogenesis, reproduction, vision, and regulation of inflammation, growth, and differentiation of normal and neoplastic cells in vertebrates.

Retinoids are alcohols and accordingly soluble in ethanol, isopropanol, and polyethylenglycol. Major sources of natural retinoids are animal fats, fish liver oil (retinylesters) and yellow and green vegetables (carotenoids). Ingested retinylesters (RE) are hydrolyzed to retinol by enteral hydrolases in the intestine. ROL and carotenoids are absorbed by intestinal mucosa cells.

Mechanism of Action

Retinoids mediate their biological effects through binding to nuclear receptors known as ►retinoic acid receptors (RARs) and ►retinoid X receptors (RXRs), which belong to the superfamily of ligand-inducible transcriptional regulators that include steroid hormone receptors, thyroid hormone receptors, and vitamin D₃ receptors [reviewed in [1]. RARs and RXRs act via polymorphic *cis*-acting responsive elements, the retinoic acid responsive elements (RAREs) and retinoid X responsive elements (RXREs), present in the promoters of retinoid-responsive genes (Fig. 1).

The known beneficial effects of retinoids on malignancies are assumed to relate to retinoid receptor-mediated antipromoting and anti-initiating effects. The latter appears to be influenced by interference of several xenobiotics with different steps of the retinoid metabolism in the target cell. Of the carotenoids, β -carotene is the most potent retinol precursor, yet being

six-fold less effective than preformed retinol, resulting from incomplete resorption and conversion (one retinol equivalent is equal to 1 μ g of retinol, 6 μ g of β -carotene, or 12 μ g of mixed carotenoids) For retinoid metabolism review see [2].

Although all-*trans*- and 9-*cis*-RA are only minor metabolites of ROL and β -carotene, they display 100–1000-fold higher biological activity. Whereas all-*trans*-RA binds only to RARs, 9-*cis*-RA binds both RARs and RXRs. The stereoisomer of all-*trans*-RA, 13-*cis*-RA, exhibits a much lower affinity for RARs and RXRs and exerts its molecular effects mostly through its isomerization into all-*trans*-RA.

Clinical Use

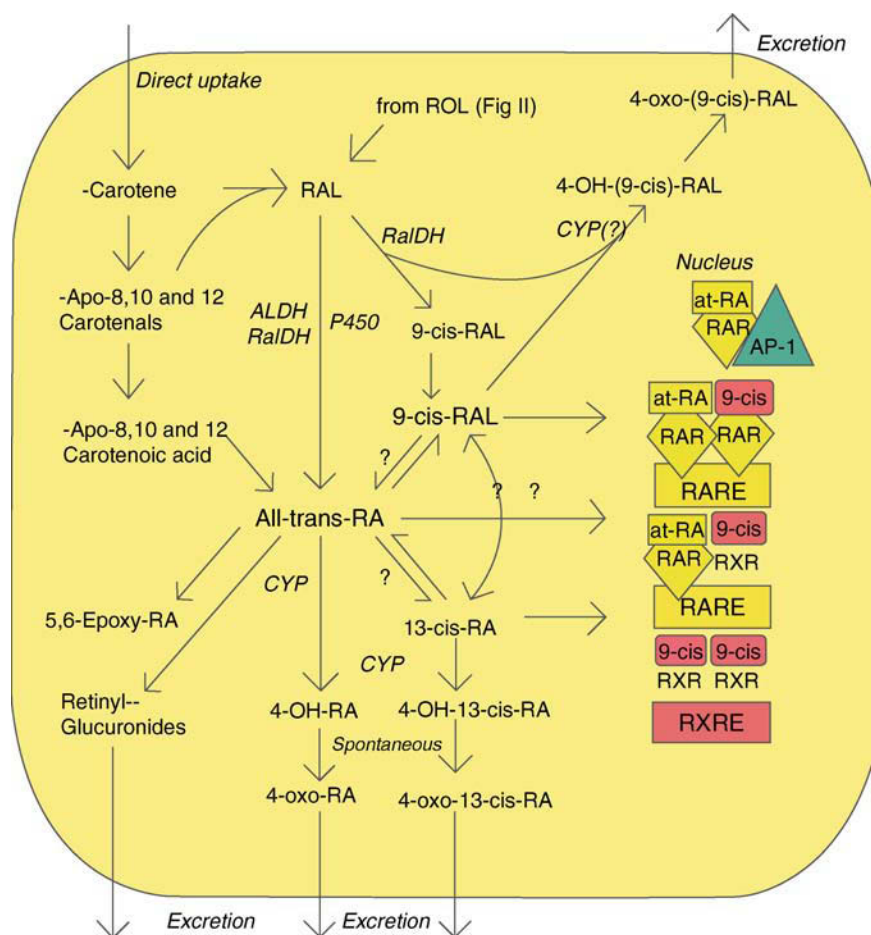
Up to now, far more than 5000 retinoic acid analogs have been synthesized, out of which the following three generations have been established for therapy of various disorders: First, the nonaromatic retinoids β -carotene (provitamin A), all-*trans*-retinoic acid (RA) (tretinoin) and 13-*cis*-RA (isotretinoin), second, the monoaromatic retinoid derivatives trimethyl-methoxyphenyl analog of RA (etretinate) and 9-(4-methoxy-2,3,6-trimethylphenyl)-3-2,4,6,8-nonatetraenoic acid (acitretin), and third, the polyaromatic retinoid derivatives tazarotenic acid and 6-[3-(1-adamantyl)-4-methoxy-phenyl]-2-naphthoic acid (adapalene) (see Table 1).

Synthetic Retinoid Receptor Selective Agonists/Antagonists

The concept of drug development is based on the findings that retinoid receptors (RARs and RXRs) offer a new approach by targeting different genes depending on the activated retinoid receptor complexes. The multiplicity of these retinoid signaling pathways affords potential for therapeutic opportunity as well as retinoid therapy associated undesired side effects. It is possible that the indiscriminate activation of all pathways by nonspecific retinoid ligands could lead to unacceptable side effects so that any enhanced efficacy would be obtained at the cost of enhanced toxicity.

The development of ligands selective for individual receptor subtypes relevant to a targeted disease could decrease these toxic effects thereby improving the therapeutic index. Two new arotinoids are already available for topical use in skin diseases. These are tazarotenic acid (tazarotene) and 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid (adapalene) (Table 1), and other synthetic retinoid derivatives are in the pipeline. Also fenretinide, a synthetic amide of retinoic acid is available for systemic therapy in oncology.

Tazarotene (Table 1) is an acetylenic third generation retinoid derivative. It is a poorly absorbed, nonisomerisable arotinoid, which is rapidly metabolized to its free carboxylic acid, tazarotenic acid, binding with high affinity to RARs, with the rank order of affinity being



Retinoids. Figure 1 Intracellular pathways and molecular action of retinoids.

$RAR\beta > RAR\gamma >> RAR\alpha$. It does not bind to any of the RXRs. This retinoid derivative is said to have lower cytotoxic effects than other retinoids while achieving sustained therapeutic efficacy in the treatment of plaque type psoriasis.

Adapalene (Table 1), a new highly stable naphthoic acid retinoid with lipophilic properties, does not bind to CRABP, although it enhances its synthesis, and its rank-order of retinoid receptor affinity appears to be $RAR\beta > RAR\gamma >> RAR\alpha$.

These retinoids, which were first introduced for the treatment of skin diseases, may also have potential as anticancer drugs. For example, the synthetic retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437) has been shown to induce apoptosis in a variety of cancer cells including lung cancer cells *in vitro*, and studies concerning the use of this agent *in vivo* would be desirable.

Future generations of such receptor subtype-selective retinoids or also retinobenzoic acids [3] may provide clinicians with more specific and less toxic drugs for dermatologic therapy.

Retinoids in Dermatology

Hyperkeratotic Disorders

The topical and oral use of retinoids for treatment of hyperkeratotic disorders such as psoriasis and Darier's disease has long been established. Systemic retinoid therapy is often combined with topical drugs such as corticosteroids, dithranol, tar, and also UVA/UVB phototherapies where synergistic effects have been reported.

Acne

Among retinoids, 13-*cis*-retinoic acid is known to have not only anti-inflammatory but also sebostatic effects. Therefore it is one of the most potent topical and also systemic agents for therapy of acne.

Photoaging and Wound Healing

Drug treatment of photoaged skin can be categorized as antioxidants, α -hydroxy acids and topical retinoids. Of these three approaches only topical retinoids, particularly retinaldehyde and all-*trans*-retinoic acid, have a well documented ability to restore the repair

function of photoaged skin at the clinical, histological and molecular level [4]. According to these observations, retinoids were also shown to accelerate wound healing [4] and reduction of early striae distensae.

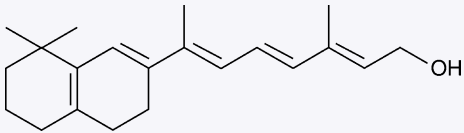
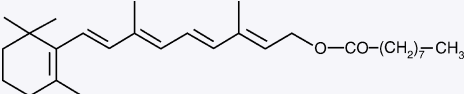
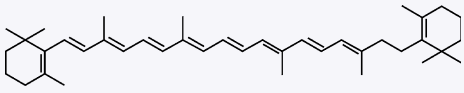
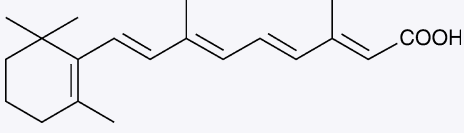
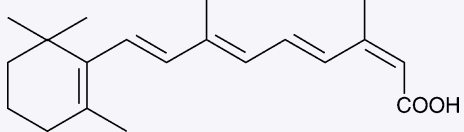
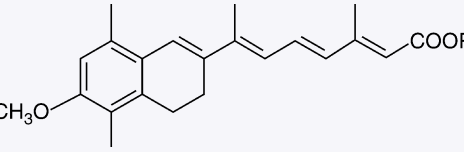
Skin and Oral Malignancies

Actinic keratoses were the first skin lesions to be treated topically with all-*trans*-retinoic acid. In various clinical trials, retinoids have been shown to be active in chemoprevention and treatment or prevention skin malignancies [2].

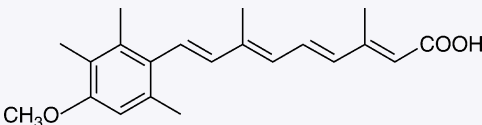
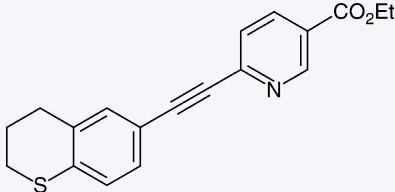
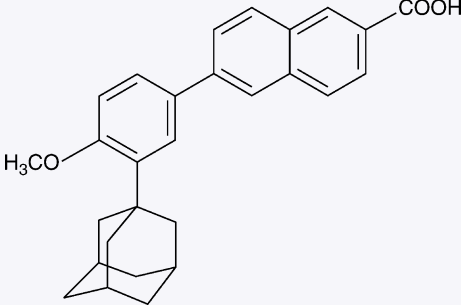
Currently, 13-*cis*-retinoic acid is the most studied chemopreventive agent that decreases the incidence of second primary tumors in patients with head-and-neck cancer, reverses premalignant lesions, and reduces appearance of nonmelanoma skin cancer in patients with xeroderma pigmentosum. Unfortunately, this vitamin A derivative has a significant clinical toxicity, which limits its utility in a practice setting.

Molecular epidemiological studies to assess the risk associated with metabolic polymorphisms for cancers of head-and-neck and the lung have shown that the overall

Retinoids. Table 1 Indications and mode of administration of commercially available retinoids in dermatological therapy

Generic name	Chemical structure	Mode of administration	Principal indication	Other indications
Retinol (vitamin A)		Oral	Vitamin supplement	
Retinyl Palmitate		Topical 0.5–5% Emulsions	Cosmetic agents	
β-Carotene (provitamin A)		Topical	Hypopigmentations, hyperpigmentations, radical protection	Nutrient color
Tretinoin		Topical 0.025–0.1% Gels or creams	Acne vulgaris, parakeratosis, hyperkeratosis	Photoaging, actinic keratosis
Isotretinoin		Topical 0.05% cream Oral 0.25–1.0 mg/kg/d	Cystic acne, recalcitrant nodular acne	Rosacea gram-negative folliculitis pyoderma faciale hidradenitis suppurativa cancer prevention
Etretinate		Oral 0.25–1.0 mg/kg/d	Generalized pustular psoriasis, exfoliative psoriasis, plaque psoriasis	

Retinoids. Table 1 Indications and mode of administration of commercially available retinoids in dermatological therapy (Continued)

Generic name	Chemical structure	Mode of administration	Principal indication	Other indications
Acitretine		Oral 0.25–1.0 mg/kg/d	Psoriasis (erythrodermic, pustular, and severe recalcitrant)	Palmoplantar keratoderma, pustulosis, palmoplantar, ichthyosis, Darier's disease, pityriasis rubra pilaris, lichen ruber planus
Tazarotene		Topical 0.05–0.1% gels	Psoriasis vulgaris	
Adapalene		Topical 0.1% gel	Acne vulgaris	

effect of common polymorphisms is moderate in terms of penetrance and relative risk. However, some gene combinations, such as mutated CYP1A1/GSTM1-null genotype, seem to predispose the lung and oral cavity of smokers to an even higher risk for cancer or DNA damage. These results require confirmation in larger studies that take into account the existence of ethnic variations even within the commonly defined groups.

Retinoids, isothiocyanates and tea polyphenols have been identified as possible chemopreventive agents for cancers of the lung and oral cavity. While a number of trials have been conducted with retinoids or β -carotene, the results were ambiguous and the causes are still being debated.

Newly acquired knowledge in the field of tumor biology and of the genetic changes underlying carcinogenesis through the use of new molecular technology represents the basis on which chemoprevention efforts should be based.

Retinoids in Oncology

Since retinoids play an important role in the molecular regulation of growth, differentiation and apoptosis of normal, premalignant and malignant cells, especially epithelial cells, numerous studies have focussed on the effect of retinoids on a variety of malignancies. In animals, vitamin A deficiency has been shown to be associated with an increased incidence of cancer and an increased susceptibility of chemical carcinogens. On the molecular level, aberrant expression and function of nuclear retinoid receptors have been found in various types of premalignant lesions and cancers. Thus, aberrations in retinoid signalling appear to be early events in carcinogenesis, and retinoids at pharmacological doses have been shown to exhibit a variety of beneficial effects associated with cancer prevention and cancer therapy e.g., by suppression of transformation, inhibition of carcinogenesis in various organs in animal models.

Lung Cancer

Epidemiological and animal studies have demonstrated that retinoids are effective agents in preventing the development of tobacco-associated cancers. Unfortunately, clinical trials of retinoids on cigarette smokers have shown lack of efficacy in preventing lung cancer. A study investigated the effect of nicotine on the anticancer activity of all *trans*-retinoic acid (*trans*-RA) in human lung cancer cells and demonstrated that nicotine could abrogate the growth inhibitory effect of *trans*-RA by suppressing its ability to induce the expression of RA receptor β (RAR β), a tumor suppressor. The inhibitory effect of nicotine was accompanied with induction of orphan receptor TR3. Inhibition of TR3 expression by overexpression of TR3 antisense RNA in H460 lung cancer cells strongly prevented the suppressive effect of nicotine on *trans*-RA activity. These results suggest that nicotine suppresses the growth inhibitory effects of all-*trans*-RA by inhibiting RAR β expression through its induction of TR3 expression. Accordingly, RXR-selective retinoids may be more effective than classical retinoids for preventing and treating tobacco-associated cancers. Another study indicates the epidermal growth factor to be a target for the lung cancer preventive effect of retinoic acid.

Encouraging findings concerning effective therapy strategies derive from combination studies in which retinoids, especially all-*trans*-retinoic acid, are added to either α -interferon or chemotherapy and radiotherapy. Here, more retinoid receptor-selective molecules may have a greater activity against lung cancer, with a more favorable toxicity profile, as recently suggested by preliminary data on Ro 41-5253 [5].

Breast Cancer

Studies with fenretinide in woman with stage I breast cancer did not show an overall effect of decreasing the risk of contralateral breast cancer. A protective effect could only be observed in premenopausal women, probably due to the modulation of the insulin-like growth factor 1 (IGF-1) by fenretinide in this population.

Liver Cancer

Hepatocellular carcinoma (HCC) develops in patients with chronic liver diseases associated with hepatitis B and hepatitis C virus infections with high incidences. Here, an acyclic retinoid has been shown to suppress the posttherapeutic recurrence after interferon- γ or glycyrrhizin treatment in cirrhotic patients who underwent curative treatment of preceding tumors. The retinoid induced the disappearance of serum lectin-reactive α -fetoprotein (AFP-L3), a tumor marker indicating the presence of unrecognizable tumors in the remnant liver, suggesting a deletion of such minute (pre)malignant clones (clonal deletion). As a molecular mechanism of the clonal deletion, a novel mechanism of

apoptosis induction by the retinoid via tissue transglutaminase has been implicated [6]. In future, a combination of immunopreventive and chemopreventive therapies may give a clue to the further advances of cancer prevention, and thereby to the improvement of the prognosis of cirrhotic patients.

Leukaemia

Acute promyelocytic leukaemia (APL) is known as the most curable subtype of acute myeloid leukaemia in adults. Here, all-*trans*-retinoic acid induces differentiation of the leukemic cells into mature granulocytes. On the basis of clinical and *in vitro* studies, the following mechanisms have been proposed to explain the frequently occurring ATRA resistance: (i) induction of accelerated metabolism of ATRA, (ii) increased expression of cellular retinoic acid-binding proteins (CRABPs), (iii) constitutive degradation of PML-RAR α , (iv) point mutations in the ligand-binding domain of RAR α of PML-RAR α , (v) P-glycoprotein expression, (vi) transcriptional repression by histone deacetylase activity, (vii) isoforms of PML-RAR α , (viii) persistent telomerase activity, and (ix) expression of type II transglutaminase. It is yet unclear which of these factors is mainly responsible for retinoid resistances. Patients, who relapse after retinoic acid therapy, should be transferred to arsenic trioxide or stem cell transplantation therapy.

Neuroblastoma

Most recently, a phase-I-study defined a dose of 13-*cis*-retinoic acid that was tolerable in patients after myeloablative therapy, and a phase-III-trial showed that postconsolidation therapy with 13-*cis*-retinoic acid improved EFS for patients with high-risk neuroblastoma [7]. Preclinical studies in neuroblastoma indicate that ATRA or 13-*cis*-RA can antagonize cytotoxic chemotherapy and radiation, such that use of 13-*cis*-RA in neuroblastoma is limited to maintenance after completion of cytotoxic chemotherapy and radiation. It is likely that recurrent disease seen during or after 13-*cis*-RA therapy in neuroblastoma is due to tumor cell resistance to retinoid-mediated differentiation induction. Studies in neuroblastoma cell lines resistant to 13-*cis*-RA and ATRA have shown that they can be sensitive, and in some cases collaterally hypersensitive, to the cytotoxic retinoid fenretinide. Here, fenretinide induces tumor cell cytotoxicity rather than differentiation, acts independently from RA receptors, and in initial phase-I-trials has been well tolerated. Clinical trials of fenretinide, alone and in combination with ceramide modulators, are in development.

Side Effects of Retinoids

Hypervitaminosis A is characterized by hepatomegaly, cerebral edema and bone structure alterations. β -Carotene

causes yellow-orange coloring of the skin by binding to keratins. Topically applied retinoic acids can lead to irritation, rash and Xerosis. Also worsening of atopic dermatitis and increased light sensitivity has been reported.

Systemic treatment of 13-*cis* retinoic acid frequently leads to cheilitis and eye irritations (e.g., unspecific cornea inflammation). Also other symptoms such as headache, pruritus, alopecia, pains of joints and bone, and exostosis formation have been reported. Notably, an increase of very low density lipoproteins and triglycerides accompanied by a decrease of the high density lipoproteins has been reported in 10–20% of treated patients. Transiently, liver function markers can increase during oral retinoid therapy. Etretnate causes the side effects of 13-*cis* retinoid acid at lower doses. In addition to this, generalized edema and centrilobular toxic liver cell necrosis have been observed.

The most important clinical side effects of systemically applied retinoic acid therapy are teratogenicity and embryotoxicity. Topical administration of retinoic acid does not appear to cause such effects. This is supported by the observation that nutritional retinoid administration can lead to higher plasma levels than topical treatment with retinoic acid. However in several countries, the topical administration during pregnancy is prohibited. In the USA, contraception during topical use of retinoic acid is recommended. This narrow therapeutic frame requires a pregnancy testing, measuring of liver enzymes, triglycerides, cholesterol and glucose before and frequent follow up examinations during retinoid therapy (every third or fourth week).

Increased risk factors for suffering retinoid side effects are adipositas, alcohol abuse, diabetes, nicotine abuse, familiar lipid metabolism alterations and other concomittant therapies (see below).

Interactions with Other Agents

Dexamethasone, the macrolide antibiotic triacetyloleandomycin, and phenobarbital are all well established inducers of the CYP3A subfamily, and can increase microsomal 4-hydroxylation of RA in rat liver. To what extent this is also the case for humans is not completely clear.

Glucocorticoids (clobetasol) also induce the expression of CYP1A1 in human skin. This is mediated through glucocorticoid receptor responsive elements (GRE) that have been identified in the first intron of the rat and human *CYP1A1* genes. These findings suggest the possibility that skin changes caused by long-term treatment with topical or systemic glucocorticoids could be mediated by a steroid-induced depletion of active retinoids. Therefore, we hypothesize that tandem treatment of patients with both glucocorticoids and low-dose RA may prevent some steroid side effects. In a mouse model this idea has already been confirmed.

Interestingly, there retinoids showed a steroid-sparing effect.

Skin procarcinogens, such as 3-MC and the polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene, can increase RA catabolism in human skin and induce local tissue depletion of retinoids, respectively. This can be antagonized by high dietary intake of β -carotene or retinoid acid. This acceleration of retinoid cleavage is primarily due to the xenobiotic-mediated induction of CYP1A1, which is also involved in the inactivation of RA to 4-OH-RA. Accordingly, retinoid-induced inhibition of basal as well as coal tar- and glucocorticoid-induced CYP1A1-expression in human skin as reported in [2] seems to reflect a competitive feedback inhibition of CYP1A1 activity by RA. Interestingly, CYP1A1 is one major enzyme that converts the procarcinogens mentioned above into active carcinogenic metabolites in skin. The induction of this enzyme, leading to an acceleration of the turnover of RA to inactive metabolites and a local RA deficiency, might further explain the profound effect of these carcinogenic CYP1A1-inducers on cell proliferation and tumor formation. In support of this notion, 7,8-benzoflavone, an inhibitor of CYP1A1 activity, increases local vitamin A concentrations, and reduces tumor formation in mouse skin [review see [2].

Imidazole antimycotics, ketoconazole, clotrimazole, and miconazole are potent inhibitors of various cytochrome P450-isoenzymes that also affect the metabolism of retinoids. They were first shown to inhibit the metabolism of RA in F9 embryonal carcinoma cells. When tested *in vitro* liarazole, a potent CYP-inhibitor, suppressed neoplastic transformation and upregulated gap junctional communication in murine and human fibroblasts, which appeared to be due to the presence of retinoids in the serum component of the cell culture medium. Furthermore, liarazole magnified the cancer chemopreventive activity of RA and β -carotene in these experiments by inhibiting RA-catabolism as demonstrated by absence of a decrease in RA-levels in the culture medium in the presence of liarazole over 48 h, whereas without liarazole 99% of RA was catabolized. *In vivo*, treatment with liarazole and ketoconazole reduced the accelerated catabolism of retinoids and increased the mean plasma all-*trans*-RA-concentration in patients with acute promyelocytic leukemia and other cancers.

Vitamin D3 (VD3) and retinoids synergistically inhibit the growth and progression of squamous cell carcinomas and actinic keratoses in chronically sun exposed skin. One reason for this synergism may be the direct influence of VD3 on the isomerization and the metabolism of RA. Here, VD3 inhibits the isomerization of 13-*cis*-RA to the more receptor active all-*trans* and 9-*cis*-isomers. Moreover, the VD3 derivative secocholestra-trien-1,3,24-triol (tacalcitol), used for the treatment of severe keratinizing disorders inhibits 4-hydroxylation of all-*trans*-RA.

Ethanol also inhibits ADH-catalyzed retinol oxidation *in vitro*, and ethanol treatment of mouse embryos has been demonstrated to reduce endogenous RA levels. The inhibition of cytosolic RoldH activity and stimulation of microsomal RoldH activity could explain ethanol-mediated vitamin A depletion, separate from ADH isoenzymes. Although the exact mechanism of inhibition of retinoid metabolism by ethanol is unclear, these observations are consistent with the finding that patients with alcoholic liver disease have depleted hepatic vitamin A reserves [review see [2].

Different combinatory therapy regimens are known which additively or synergistically act in a variety of diseases:

Combinations of drugs displaying distinct effects on cell proliferation/ differentiation and immunomodulation (e.g., retinoids and chemotherapy in advanced cutaneous T cell lymphoma).

Combinations of retinoids with ultraviolet A or B radiation (and other drugs). For example, RePUVA-therapy (retinoids and psoralen and UVA combination) is currently one of the most effective regimens for recalcitrant severe psoriasis.

Drugs with metabolic interactions that can enhance the half-life of active compounds. An example of this regimen is the interaction between azole- and vitamin D-derivatives that inhibit the metabolism of retinoids in skin cells leading to increased intracellular amounts of active RA-isomers. Further study and the identification of novel interactions of this type of drug interaction is of great clinical interest since they may decrease the dose of retinoids required for efficacy thereby also reducing the risk of side effects of the retinoids.

Retinoid-Resistance

(►Retinoid-resistance) Two possible explanations for accelerated clearance of retinoids in patients during long-term treatment with retinoids have been suggested: First, RA-mediated induction of CRABP-expression, which is known to lower the plasma and intracellular levels of active RA by binding RA, and second, the RA-mediated induction and/or constitutive overexpression of P-glycoprotein, which is encoded by the multidrug resistance gene-1, leading to decreased intracellular levels of RA by enhancing active transport of intracellular retinoids out of the target cells [review see [2].

The knowledge concerning the molecular action of retinoids is steadily increasing but still the many steps of retinoid metabolism especially retinoid inactivation are not fully understood. The interaction of retinoids as the central agent with other drugs represents a new dimension of disease therapy providing us with more specific and less toxic therapy approaches to influence cell proliferation and differentiation. Perhaps in no other area of pharmacology is the concept of using drug–drug interactions as a rationale for therapy more

advanced than with retinoids in dermatology. It is likely that this strategy will prove useful in other areas as well.

►Glucocorticoid Receptors

►Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

►Retinoids

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Retinol

►Vitamin A

►Retinoids

Retinyl Esters

►Vitamin A

Retrograde Messenger

A neuromodulator that is released from the post-synaptic neuron and acts at the pre-synaptic neuron that makes a

synapse with the post-synaptic neuron. Retrograde messengers are involved in synaptic plasticity.

- Endocannabinoids

Retroviruses

- Gene Therapy Vectors
- Antiviral Drugs

Reuptake Transporter

Reuptake transporters are structures within the cell membranes of the presynaptic nerve terminal that serve to transport biogenic amines released from vesicles back into the nerve cell. These structures are targets for antidepressants, which block the transporter, thus increasing the bioavailability of neurotransmitters at postsynaptic receptors.

- Antidepressant Drugs
- Neurotransmitter Transporters

Reverse Complementary Oligonucleotides

- Antisense Oligonucleotides (ASON)

Reverse Mode

Mode of operation of the sodium calcium exchanger extruding sodium and intruding calcium ions.

- $\text{Na}^+/\text{Ca}^{2+}$ Exchangers

Reverse Pharmacology

A scientific procedure that turns the classical pharmacology approach “upside down.” Instead of finding the elusive receptor for a known hormone or transmitter what classical pharmacology aims at, reverse pharmacology is initiated through the discovery of the receptor gene and aspires to the identification of the receptors unknown ligand.

- Orphan Receptors

Reverse Transcription

Reverse transcription is the copying of an RNA molecule back into its DNA complement. The enzymes that perform this function are called reverse transcriptases. Reverse transcription is used naturally by retroviruses to insert themselves into an organism’s genome. Artificially induced reverse transcription is a useful technique for translating unstable messenger RNA (mRNA) molecules into stable cDNA.

- Antiviral Drugs
- Viral Proteases

Reward (Pathways)

- Drug Addiction/Dependence
- Dependence
- Psychostimulants

Reye's Syndrome

Reye syndrome is a rare disorder in children, characterized by a combination of severe liver disorder and encephalopathy (central nervous system (CNS) disturbances) that can follow an acute viral illness and which has a relatively high mortality. It has been found to be

associated with the use of acetylsalicylic acid (aspirin). Although it is not completely clear to what extent aspirin is in fact implicated in the pathogenesis of the syndrome, aspirin is best avoided in children with viral infections.

► **Non-steroidal Anti-inflammatory Drugs**

RGS Protein

Regulators of G-protein signalling.

- **Table appendix: Receptor Proteins**
- **G-Proteins**

Rhabdomyolysis

Rhabdomyolysis is disintegration and death of muscle cells (myocytes). It is an important but rare side effect of treatment with statins.

- **HMG-CoA-Reductase Inhibitors (Statins)**

Rheumatoid Arthritis

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Definition

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown aetiology with some autoimmune features. Current thinking favours the hypothesis that interplay between genetic factors, sex hormones, and possibly an infectious agent or another immune activating agent initiates an autoimmune pathogenic mechanism that culminates in a disease with inflammatory and destructive features.

To date there is no diagnostic test specific for rheumatoid arthritis. Revised classification criteria for the diagnosis of RA were formulated by the American College of Rheumatology [1] based on a hospital

population of patients with established, active disease. These criteria combine a constellation of clinical, serological and radiological features and have become widely accepted for epidemiological and clinical studies. By emphasising key features of the disease, the criteria help to differentiate RA from other forms of inflammatory arthritis with a diagnostic sensitivity and specificity of about 90% for active disease. However, these requirements have a much poorer sensitivity for a diagnosis of RA in the early stages of presentation at which time there may be an insufficient number of features required to satisfy the classification criteria.

Basic Mechanisms Characteristics

Once established, RA is characterised by deforming symmetrical polyarthritis associated with synovitis of joint and tendon sheaths, articular cartilage loss and erosion of juxta-articular bone of varying extent and severity [2]. IgM ► **rheumatoid factor** is detectable in the blood in a majority of patients. It is an autoantibody whose autoantigen is the Fc portion of IgG. The prevalence of rheumatoid factor increases with duration of disease in rheumatoid arthritis: at 3 months the prevalence is 33%, while at 1 year it is 75%. Up to 20% of RA patients remain negative for rheumatoid factor (also known as “seronegative rheumatoid arthritis”) throughout the course of their disease. ► **Antibodies to cyclic-citrullinated peptides** (anti-CCP) have a similar sensitivity to rheumatoid factor, but higher specificity [3]. As in the case of high-titre rheumatoid factor, anti-CCP antibodies are associated with persistence and destructiveness of rheumatoid arthritis and may precede the onset of clinical disease.

In a proportion of patients systemic and extra-articular features may be observed during the course of the disease (and rarely prior to joint disease). These include anaemia, weight loss, vasculitis, serositis, nodules in subcutaneous, pulmonary and sclera tissues, mono-neuritis multiplex, and interstitial inflammation in lungs as well as in exocrine salivary and lacrimal tissue. However, these systemic manifestations occur relatively late in the disease progression.

The clinical presentation of RA is heterogeneous with a wide spectrum of age of onset, degree of joint involvement and severity. Similarly, the disease course of RA is variable. This ranges from a brief, mild, self-limiting oligoarticular illness with minimal joint damage to a sustained polyarticular, synovial inflammation resulting in relentlessly progressive cartilage destruction, erosion of bone and ultimately changes in joint integrity with corresponding functional impairment. Up to 90% of patients with marked synovitis have radiographic evidence of bone erosion within 2 years of diagnosis, despite treatment with traditional disease modifying agents (such as methotrexate,

sulphasalazine and gold). The majority of patients with a more aggressive disease evolution become clinically disabled within 20 years (Fig. 1). For those with severe disease or extra-articular features the mortality is equivalent to that of patients with three vessel coronary artery disease or stage IV Hodgkin's lymphoma.

Cellular Pathology

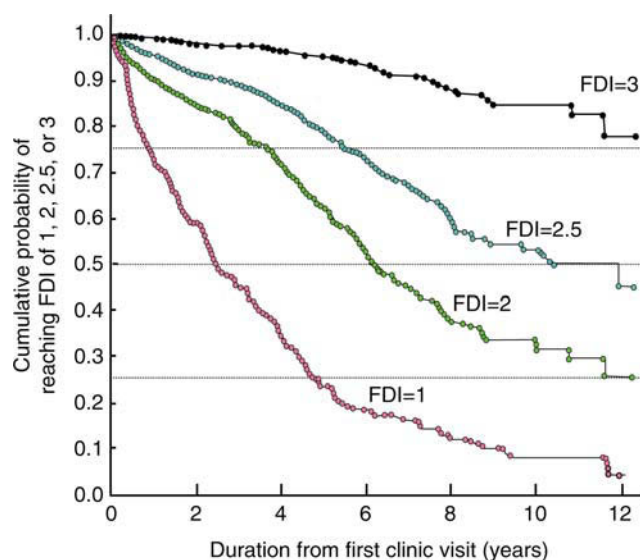
RA is characterised by chronic inflammation of synovial joints with synovial proliferation and infiltration by blood derived cells, in particular, memory T cells, macrophages and plasma cells, all of which show signs of activation. Prominent vasculature is a feature of RA synovitis that is observed as a fine network of vessels over the rheumatoid synovium at arthroscopic inspection of RA joints. Angiogenesis is evident on microscopic examination of synovial biopsies from the earliest stages of disease development. Formation of new blood vessels permits a supply of nutrients and oxygen to the augmented inflammatory cell mass and so contributes to the perpetuation of synovitis. In the chronic phase of disease, capillaries and post-capillary venules are particularly evident in the synovial sublining region. In histological sections mononuclear and polymorphonuclear leukocytes can sometimes be found in close apposition to vascular endothelium, probably in the process of margination and adhesion prior to migration into the inflamed tissue. The synovial tissue becomes markedly hyperplastic and locally invasive at the interface of cartilage and bone with progressive destruction of these tissues in the majority of cases. This invasive tissue is referred to as 'pannus', comprising mainly lining cells with the appearance

of proliferating mesenchymal cells with very little sublining lymphocytic infiltration. The accompanying destruction of bone and cartilage is thought to be mediated by cytokine-induced degradative enzymes, notably the matrix metalloproteinases. Although RA has its principal manifestation in joints there is also evidence of systemic involvement, for example the upregulation of acute phase proteins, and a wide variety of extra-articular features may develop. These occur predominantly in patients who are rheumatoid factor positive and carry the HLA-DR4 gene.

Genetic Factors

Genetic factors were originally implicated in the aetiopathogenesis of RA following the discovery that in population studies there is a slight increase in the frequency of RA in first-degree relatives of patients with this disease, especially if seropositive for rheumatoid factor. In hospital-based population studies of identical twins, concordance rates of disease are around 30%, compared with 5% in non-identical twins. The figures are lower in community-based studies and although still supportive of the concept of a genetic contribution, argue against the proposition that RA is the result of a dominant single-gene disorder. These and other epidemiological studies have led to the conclusion that RA is a polygenic disease, and that non-inherited factors are also of great importance [3].

Genes encoding particular class II ▶**Human Leucocyte Antigens** (HLA) are among candidates for involvement in predisposition to RA. This discovery came about with the observation that 60–70% of Caucasian patients with RA are HLA-DR4 positive by



Rheumatoid Arthritis. Figure 1 The development of disability over time in a group of RA patients studied prior to the early 1990s. FDI = functional disease index where 1 = moderate disability, 2 = more marked disability, 3 = severe disability and 4 = very severe disability (from Wolfe, Cathey (1991) *J Rheumatol* 18:1298).

cellular or serological techniques compared with 20–25% of control populations. Furthermore, patients with more severe RA, especially those with extra-articular complications such as vasculitis and Felty's syndrome, are even more likely to be HLA-DR4 positive than patients with less severe disease confined to joints.

Class II HLA molecules are expressed on the surface of antigen-presenting cells. They play a key role in presentation of processed linear peptide antigens of at least nine amino acids to T cells. Antigen is bound to the HLA antigen binding cleft formed by the α and β chains of the HLA class II molecule. This tri-molecular HLA–antigen complex binds in turn to the variable portion of the T-cell receptor.

Nucleotide sequencing of HLA-DR β ₁ exons coding amino acid residues 70–74 has revealed that HLA-DR4 subtypes Dw4, Dw14, and Dw15 share similarities with each other (with a conservative substitution of glutamine with lysine at position 71 in Dw4) and with HLA-DR1. The sequence predicts susceptibility to RA and is associated with RA in 83% of Caucasian patients in the United Kingdom. In contrast, negative associations are observed in individuals who are DR4w10, in whom the charged basic amino acids glutamine and arginine in positions 70 and 71 are replaced by the acidic amino acids aspartic and glutamic acid. In Dw13 individuals, in whom a negative association is also observed, arginine is substituted for glutamic acid in position 74. Molecular modelling studies suggest that amino acid residues 70–74 are located in the α -helix forming the wall of the peptide-binding groove, and thus likely to be involved in antigen binding and subsequent interaction with T-cell receptors. Acidic substitutions could profoundly alter protein structures and thereby alter affinity for peptide antigens. However, molecular mechanisms accounting for susceptibility to RA remain to be elucidated. Possibilities include permissive binding of specific peptides such as those on autoantigens or on environmental antigens, initiation of disease by specific binding of superantigens to HLA molecules, or modulation of the T-cell repertoire by selection or tolerance. It has been hypothesized that the severity of disease and extra-articular complications are related to homozygosity and the density of disease-associated MHC molecules that critically influence the selection of the T-cell repertoire and tolerance to antigens.

Cytokines

Cytokines are small, short-lived proteins and important mediators of local intercellular communication. They play a key role in integrating responses to a variety of stimuli in immune and inflammatory processes. By binding their cognate receptors on target cells in their immediate vicinity, these molecules participate in many important biological activities including cell proliferation, activation, death and differentiation. In

experimental systems, some cytokines are proinflammatory, such as interleukin-1 (IL-1) and TNF α ; others, such as interleukin-10 (IL-10) and transforming growth factor β (TGF β) exert predominantly anti-inflammatory effects. However, it is now known that many cytokines, for example, interferon γ (IFN- γ), with chiefly proinflammatory activity can also in some instances have anti-inflammatory properties. Similarly, IL-10 and TGF β may also exhibit proinflammatory properties under certain experimental conditions. Interleukin-6 (IL-6) does not have a classical proinflammatory action but has been implicated in the process of erosion of bone in inflammatory arthritis. ►**Paracrine** or ►**autocrine** pathways involving cytokines with either pro- or anti-inflammatory activity form complex networks determining whether chronic inflammation results.

Role of Cytokines in the Pathogenesis of RA

Cytokines derived from macrophages and fibroblasts are abundant in the rheumatoid synovium. These include IL-1, TNF α , granulocyte macrophage colony stimulating factor (GM-CSF), IL-6, and numerous chemo-attractant cytokines known as chemokines [4]. Many of these factors are of importance in regulating inflammatory cell migration and activation. By contrast, given the extent of synovial inflammation and lymphocytic infiltration, factors produced by T cells, for example, IFN γ , interleukin-2 (IL-2) and interleukin-4 (IL-4) are surprisingly sparsely expressed. However, there are a number of cytokines that cause ►**co-stimulation** of T helper cells including interleukin (IL)-7, IL-12, IL-15 and IL-18. There is a predominance of Th1 cell activity, as defined by IFN γ production, and low Th2 cell activity as defined by IL-4 production.

An extensive range of proinflammatory cytokines can be detected in RA synovial samples, regardless of differences in donor disease duration, severity or even conventional (non-biologic) drug therapy. Proinflammatory cytokines are spontaneously produced over several days in dissociated RA synovial membrane cell cultures comprising a heterogeneous population of cells. This occurs in the absence of extrinsic stimulation, suggesting that the cultures produce one or more soluble factors regulating prolonged cytokine synthesis. Addition of anti-TNF antibodies to these cell cultures was observed to strikingly reduce the production of other proinflammatory cytokines, including IL-1, GM-CSF, IL-6 and IL-8. In contrast, blockade of IL-1 by means of the IL-1 receptor antagonist results in reduced production of IL-6 and IL-8 but not that of TNF α . These observations led to the concept that TNF α occupies a dominant position at the apex of a proinflammatory cytokine network.

In support of the concept of a cytokine dysequilibrium within the chronic inflammatory situation in rheumatoid synovium is the observation that multiple

anti-inflammatory mediators are also upregulated, but at a level insufficient to suppress synovitis. Examples include the abundant expression of IL-10, IL-13 and TGF β both in latent and active form. Naturally occurring cytokine inhibitors, such as interleukin-1 receptor antagonists (IL-1ra) and soluble TNF receptors, the specific inhibitors of IL-1 and TNF respectively, are also upregulated in the rheumatoid joint (Fig. 2).

TNF α is a pleiotropic cytokine with biological properties that include enhanced synovial proliferation, production of prostaglandins and metalloproteinases as well as regulation of other proinflammatory cytokines. The predicted clinical success of anti-TNF therapy in RA was based on the demonstration of RA synovial tissue expression of TNF α and its receptors, in vitro experiments employing dissociated synovial cell cultures and pre-clinical in vivo studies [4]. A number of independent in vivo studies demonstrated that antibody therapies blocking bioactivity of TNF α , administered either during the induction phase of murine collagen-induced arthritis or, more importantly, after the onset of disease, were able to ameliorate clinical symptoms and prevent joint destruction. Furthermore, in a murine model, the over-expression of a human TNF α transgene modified at its 3' end to prevent degradation of its mRNA was associated with the development of a destructive form of polyarthritis 4–6 weeks after birth. This could be prevented by administration of a human TNF-specific mAb.

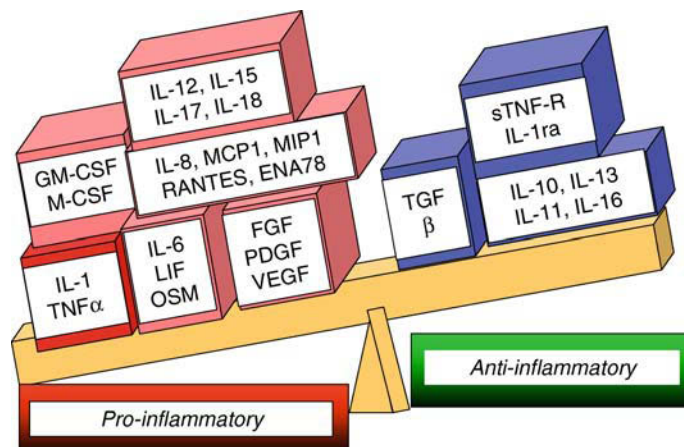
Pharmacological Intervention

The treatment approach to RA has undergone a major evolutionary change in recent years with a move away from predominantly symptomatic treatment approaches towards much earlier intervention with ►[disease-modifying anti-rheumatic drug](#) (DMARD) therapy and

the use of treatment regimes designed to optimally suppress synovitis at any given stage of disease [2]. This is a consequence of a growing appreciation of the severity of this condition, better understanding of the most effective use of conventional DMARDs and advances in development of biologic treatments. Conventional DMARDs include low dose once weekly methotrexate, sulphasalazine, hydroxychloroquine and leflunomide. Corticosteroids are often used as a “bridging” therapy when a more slowly acting oral DMARD is initiated so that rapid suppression of synovitis can be achieved before the slower benefits of the DMARD are expressed. ►[Biologics](#) are protein-based drugs derived from living organisms that are designed to either inhibit or augment specific component of the immune system. The major impetus for development of biologic therapies has come from advances in molecular technology that have facilitated identification of cell subsets and cytokines contributing to the inflammatory and destructive components of the disease, prompting development of specific targeted therapies directed at relevant disease molecules [5].

Very considerable progress in understanding the important role of cytokines in the immunopathogenesis of RA has led to two potential approaches to cytokine modulation of rheumatoid synovitis: inhibition of dominant proinflammatory cytokines such as TNF α , IL-1, IL-6 or IL-15, or augmenting the inadequate anti-inflammatory activity of certain cytokines or naturally occurring cytokine inhibitors as, for example, by administration of soluble TNF receptors or IL-1ra. It is now established that the long-term use of biological agents targeting TNF α gives rise to sustained improvements in symptoms and signs of rheumatoid disease.

Anti-TNF therapy, particularly when used in combination with methotrexate, gives rise to substantial



Rheumatoid Arthritis. Figure 2 The concept of a cytokine dysequilibrium. Many cytokines are detectable in rheumatoid synovial tissues, including those with predominantly anti-inflammatory properties. But the net effect is a dominance of proinflammatory activity.

improvements in symptoms and signs of disease in about two-thirds of RA patients. Furthermore, this treatment combination protects joints from structural damage in a majority of patients. Furthermore, this can occur irrespective of whether a clinical response is achieved or not [2].

Although anti-TNF α agents are well tolerated and have a good overall safety profile, pitfalls to the use of these drugs apparent with increasing clinical experience include infective complications and, in particular, reactivation of tuberculosis. To date, no statistically significant increased rate of tumour occurrence over that expected has been noted although cases of lymphoma have rarely been reported in patients treated with TNF α blockade.

Clinical trials of IL-1 receptor antagonist show relatively modest anti-inflammatory efficacy but radiographic evidence indicative of retardation of joint damage. Several other proinflammatory cytokines represent potential therapeutic targets including IFN- β , IFN- γ , IL-6, IL-15, IL-17 and IL-18 and biological interventions targeting these molecules are in clinical trials. For example, data for blockade of the biological effect of IL-6 by means of intravenously administered antibodies to the IL-6 receptor added to background methotrexate therapy demonstrate efficacy for reduction in symptoms and signs with acceptable safety.

In contrast to the dramatic success of TNF blockade as a new approach to RA therapy, early randomised, placebo-controlled clinical studies exploring the potential of biological therapies targeting T cells in the treatment of RA have had generally disappointing results. Some anti-T cell agents were non-efficacious whereas other preliminary trials demonstrating some clinical efficacy were terminated due to adverse events, particularly prolonged and profound T cell depletion. However, the primatized monoclonal anti-CD4 antibody keliximab results in dose-dependent clinical responses when administered once weekly over 4 consecutive weeks and the clinical response correlates with CD4⁺ T cell coating with keliximab rather than T cell depletion.

An alternative approach seeks not to deplete or inactivate T cells, but to modulate their function in such a way as to reduce their pathogenicity. For example, the co-stimulation blocker abatacept (CTLA4-Ig), which blocks the interaction between the B7 receptor on antigen presenting cells and CD28 on T cells, has efficacy in RA patients with an inadequate response to methotrexate although in general, it takes longer for the maximum benefit to be achieved as compared with anti-TNF agents.

The potential of B lymphocyte depletion as an approach to therapy has been confirmed in RA patients seropositive for rheumatoid factor and/or anti-CCP antibodies using the anti-CD20 mAb, rituximab.

In summary, recent years have seen the emergence of encouraging data for antibodies in clinical development that are directed against proinflammatory cytokines, inflammatory cells or co-stimulatory molecules. In particular, anti-TNF α therapies have set a new standard for symptom control and prevention of joint destruction in RA.

- Chemokine Receptors
- Immunosuppressive Agents
- Non-steroidal Anti-inflammatory Drugs
- Glucocorticoids
- Cyclooxygenases

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Rheumatoid Factor

It is an autoantibody whose autoantigen is the Fc portion of IgG. Rheumatoid factors may be of any immunoglobulin isotype but it is IgM rheumatoid factor that is commonly measured in rheumatoid arthritis. Classification criteria for rheumatoid arthritis include only one serological test, namely rheumatoid factor. However, it is not diagnostic test; rather it may be confirmatory when a number of other clinical features are present.

- Rheumatoid Arthritis

Rho

A small monomeric GTPase that regulates a number of cellular functions.

- Smooth Muscle Tone Regulation
- Small GTPases

Rho-kinase

The direct target for Rho. The enzyme is inhibited by Y-27632, a compound that lowers elevated blood pressure in animal models of hypertension.

► Smooth Muscle Tone Regulation

Riboflavin

Riboflavin is vitamin B2.

► Vitamin B2

Ribosomal Protein Synthesis Inhibitors

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Synonyms

Translation inhibitors; Ribosome-targeting antibiotics

Definition

The ribosome is the cellular target of a large and chemically diverse group of antibiotics. The antibiotic binding sites are clustered at functional centers of the ribosome and the majority are composed exclusively of RNA. The drugs interfere with the positioning and movement of substrates, products and ribosomal components that are essential for protein synthesis.

Basic Characteristics

Ribosomes and Protein Synthesis

Ribosomes are ancient ribonucleoprotein complexes that are the sites of protein synthesis in living cells. Their core structures and fundamental functional mechanisms have been conserved throughout the three domains of life: bacteria, archaea and eukaryotes. All ribosomes are organized into two subunits that are defined by their apparent sedimentation coefficient, measured in Svedberg units (S). There is a general

increase in ribosome size with organism complexity from bacteria to archaea to eukaryotes. The smaller bacterial ribosome has a molecular mass of approximately 2.5 MD and is composed of a 30S small subunit, with 16S ribosomal RNA (rRNA; 1,540 nucleotides) and 21 proteins, and a 50S large subunit, with 23S rRNA (2,900 nucleotides), 5S rRNA (120 nucleotides) and over 30 proteins. Progress in the structural elucidation of the ribosome through X-ray crystallography has resulted in atomic resolution structures of ribosomal subunits and their complexes in association with antibiotics. These structures reveal molecular details of antibiotic binding to the ribosome and insights into the structural basis for antibiotic inhibition and resistance [1].

Protein synthesis is a complex multi-step process that can be divided into initiation, elongation and termination stages. In the initiation phase, the 30S subunit binds to messenger RNA (mRNA), along which triplet base codons specify the individual amino acids in a protein sequence that will be added via cognate transfer RNAs (tRNAs). tRNAs span the interface between the subunits, with the tRNA anticodon and aminoacylated ends interacting with the 30S and 50S subunits, respectively. The tRNA carrying the first amino acid (methionine) binds the mRNA on the 30S subunit through codon-anticodon base pairing, followed by positioning of the 3'-end in the peptidyl-donor site in a cavity on the 50S subunit. The second tRNA binds at the aminoacyl-acceptor site with its 3'-end positioned next to that in the donor site. Peptide bond formation occurs, catalyzed by the 50S subunit and resulting in transfer of the nascent peptide to the aminoacylated tRNA. The precise positioning of the 3'-ends of aminoacyl- and peptidyl-tRNA substrates is achieved in part through base pairing interactions with the A-loop and P-loop of 23S rRNA, respectively. Although the mechanism of peptidyl transferase remains unclear, the atomic resolution structure of the archaeon *Haloarcula marismortui* 50S subunit complexed with a transition state analog reveals that there are no ribosomal proteins within 18 Å of the nascent peptide bond [2]. Peptide bond formation is followed by translocation of the mRNA-tRNA complex, leaving a vacant A-site for the tRNA carrying the next encoded amino acid. Incoming tRNAs move through successive sites on the ribosome in a unidirectional manner, including aminoacyl-acceptor (A), peptidyl-donor (P) and exit (E) sites. This elongation cycle is repeated for each encoded amino acid. Termination occurs upon recognition of a termination codon, which triggers release of the nascent polypeptide along an exit channel through the 50S subunit, followed by subunit dissociation. The entire process is aided by protein factors at every stage, including aminoacyl-tRNA selection and translocation of the mRNA-tRNA complex.

The Ribosome as a Target for Antibiotics

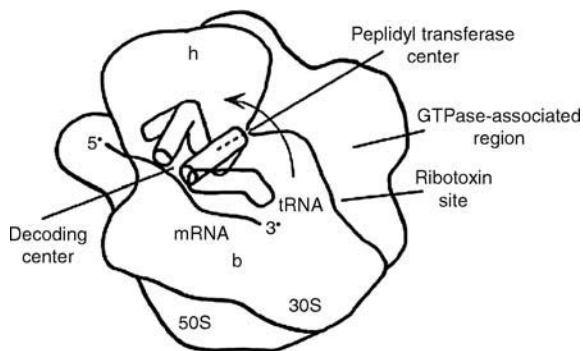
The ribosome is an important target for a wide variety of antibiotics. Antibiotics inhibit protein synthesis at different functional steps and have served as valuable tools in determining the mechanisms of translation. Although a few universal antibiotics inhibit protein synthesis in most, if not all, living organisms, the majority of antibiotics exhibit selectivity for ribosomes from one or two domains of life. Consequently, many of the drugs have important medical applications in the treatment of serious bacterial infections. The wide clinical use of some antibiotics, however, has been curtailed in recent years due to problems of toxicity and antibiotic resistance. A detailed understanding of antibiotic inhibitory mechanisms has remained elusive despite the fact that many of the drugs have been known for decades. An important step forward has come with the breakthroughs in ribosome crystallography and the structural elucidation of ribosomal subunit-antibiotic complexes, obtained from crystals of ribosomal subunits through either soaking or co-crystallization with antibiotics. These complexes localize antibiotic binding sites and yield molecular insights into the structural basis of antibiotic inhibition and resistance.

A number of general points can be made on antibiotic targeting of the ribosome: First, antibiotics bind to specific sites on the ribosome, with the majority targeting ribosomal RNA and not ribosomal proteins. This is consistent with crystallographic data showing that many functional centers on the ribosome are composed largely of rRNA. In addition, many antibiotics bind to regions of rRNA that are near mRNA template or tRNA substrate binding sites. Finally, antibiotics bind to regions of the ribosome that undergo conformational changes or rearrangements during translation. Most antibiotics that target the ribosome act at or near one of the following functional centers (Fig. 1). These are (i) the decoding center on 16S rRNA, (ii) the GTPase-associated region, (iii) the ribotoxin site and (iv) the ▶peptidyl transferase center, where the latter three involve 23S rRNA. This review will focus on antibiotics that target functional centers (i) and (iv), on which there is high-resolution structural information available from ribosomal subunit-antibiotic complexes.

Drugs

Antibiotics and the 30S Subunit

The two primary functions of the 30S subunit in protein synthesis are (i) decoding, the discrimination between cognate and non-cognate tRNAs by monitoring codon-anticodon base pairing in the ribosomal A site, and (ii) translocation, where, together with the 50S subunit, tRNAs and the associated mRNA are moved by precisely one codon. The high degree of translational



Ribosomal Protein Synthesis Inhibitors.

Figure 1 Schematic drawing of the morphology of the ribosome. The ribosomal subunits are labeled, as are the approximate locations of their respective functional centers. The drawing is a transparent view from the solvent side of the small subunit. Transfer RNAs are shown in different binding states with the arrow indicating their direction of movement through the ribosome. The tRNA anticodon ends are oriented towards the viewer, whereas the 3'-ends of the tRNAs are oriented towards the peptidyl transferase region on the large subunit. The letters "h" and "b" denote the head and body regions on the 30S subunit, respectively.

accuracy achieved by the ribosome is thought to involve both initial selection (via codon-anticodon base pairing) and proofreading steps, with the latter important for discrimination between cognate and near-cognate tRNAs. The structures of bacterial 30S-antibiotic complexes yield insights into how the following drugs interfere with 30S function: spectinomycin, streptomycin, paromomycin, tetracycline and pactamycin [3, 4].

Spectinomycin inhibits translocation of peptidyl-tRNA from the A- to the P-site. The antibiotic has a rigid structure composed of three fused rings that binds at one end of helix 34 in the minor groove, interacting mostly with nucleotides G1064 and C1192. Translocation requires movement of elements of the head of the 30S subunit, including helix 34. Through binding near a pivot point of the head, spectinomycin can interfere with movements of the head through steric hindrance and thereby block translocation.

Streptomycin is thought to make ribosomes error-prone by affecting proofreading and initial selection steps. The antibiotic interacts with four points of the phosphate backbone of 16S rRNA (at nucleotides 13, 526, 915 and 1,490) through salt bridges and hydrogen bonds. It also contacts lysine 45 of protein S12. The binding site suggests that streptomycin affects the helix 27 accuracy switch by stabilizing the *ram* state of the ribosome. In the *ram* state there is a higher affinity for tRNA in the A-site, which increases binding of non-cognate tRNAs. Stabilization of the *ram* state would make switching to the restrictive state more difficult and

thereby affect proofreading. A number of streptomycin-resistance mutations in 16S rRNA and protein S12 result in a hyperaccurate phenotype. In general, these mutations disrupt interactions that contribute to stabilization of the *ram* state.

Paromomycin belongs to a subclass of aminoglycoside antibiotics that target an asymmetric internal loop element in helix 44 of 16S rRNA, where they decrease the fidelity of translation. These antibiotics contain a 2-deoxystreptamine ring and an aminoglycoside ring that is either 4,5-disubstituted (neomycin and paromomycin) or 4,6-disubstituted (gentamycin and kanamycin). Paromomycin binds in the major groove of helix 44, where rings i and ii direct the specific interaction with 16S rRNA (Fig. 2). Ring i stacks against G1491 and hydrogen bonds to A1408 and the backbone of A1493, to flip A1492 and A1493 out of the helix. The unstacked bases point into the A-site and are positioned to interact in the major groove of the codon-anticodon helix. The bases hydrogen bond to 2'-hydroxyl groups on both sides of the codon-anticodon helix and are thereby able to monitor the shape and width of the minor groove of three consecutive base pairs, allowing for discrimination between correct base pairing and mismatches. Structural data suggests that unstacking of A1492 and A1493 occurs during normal translation if favorable interactions can be made across the minor groove with both mRNA and

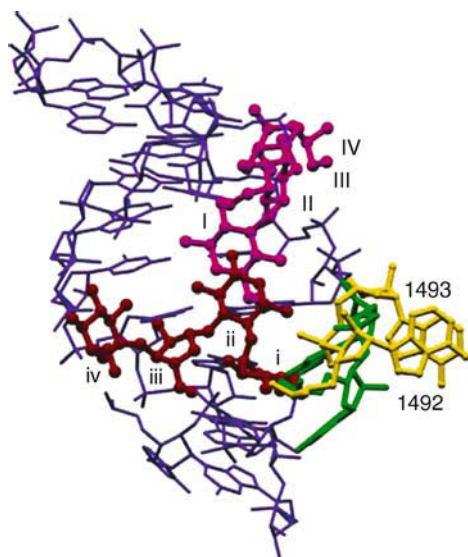
cognate tRNA in the codon-anticodon helix. When paromomycin is bound, the bases are flipped out of the helix causing an increase in the affinity of cognate and near-cognate tRNAs, and also the error rate. NMR studies show that paromomycin and gentamycin interact with 16S rRNA in the same manner, indicating that other aminoglycosides induce errors in translation via the same mechanism.

Hygromycin B is an aminoglycoside composed of four rings (I–IV) that inhibits translocation by sequestering tRNA in the A-site. It binds above paromomycin in the major groove near the top of helix 44 (Fig. 2), contacting rRNA from both strands (nucleotides 1,400–1,410 and 1,490–1,500). Hygromycin B binds exclusively to RNA bases in a sequence-specific manner. The drug adopts an extended structure and makes base-specific hydrogen bonds spanning more than three bases along one strand of helix 44. Hygromycin B prevents movement from the A- to the P-site by interacting with regions of helix 44 that are known to be involved in translocation. Binding of the drug could also disrupt the switch between *ram* and restrictive states since this transition affects bases in the hygromycin B binding site.

► **Tetracycline** is known to block binding of aminoacyl-tRNA to the A-site, but not initial binding of the EF-Tu:aminoacyl-tRNA:GTP ternary complex. The primary binding site is located just above the binding site for aminoacyl tRNA, between the head and the body of the 30S subunit. The drug interacts with the sugar phosphate backbone of residues in helices 31 and 34 through hydrogen bonds with oxygen atoms and hydroxyl groups on the hydrophilic side of the drug (Fig. 3). A magnesium ion is found in the binding site that mediates salt bridge interactions between the drug and the backbone of 16S rRNA. The binding site is not well conserved between bacteria and eukaryotes, providing a rationale for the specificity of the drug for bacteria. Tetracycline prevents binding of tRNA in the A-site through steric hindrance. The initial binding of tRNA in the ternary complex is not affected since its angle of approach is different from free tRNA in the A-site, avoiding a steric clash with the antibiotic.

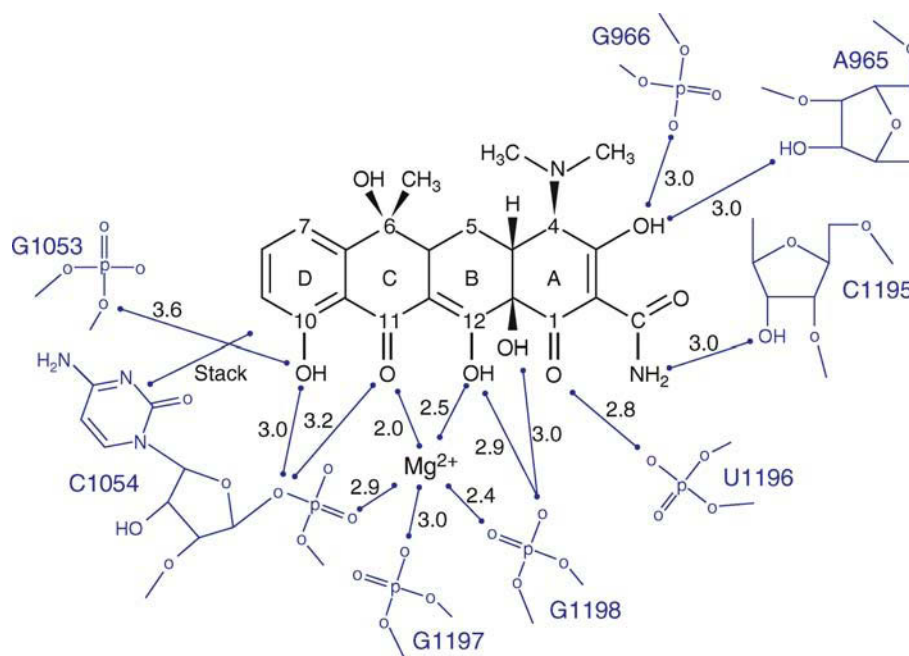
Tetracycline has a secondary binding site in the H27 switch region that may also be functionally significant. The drug binds at the interface of the three domains of 16S rRNA, close to helix 44 and between helices 11 and 27. As with the primary binding site, contacts are made from the hydrophilic face of the drug to the backbone of 16S rRNA. In this binding site, tetracycline may function to stabilize the *ram* state.

The universal antibiotic pactamycin targets a highly conserved region of 16S rRNA, contacting the tips of helices 23b and 24a in the central domain. Pactamycin folds up to mimic a RNA dinucleotide in that its



Ribosomal Protein Synthesis Inhibitors.

Figure 2 The bound structures of paromomycin and hygromycin B in helix 44 of the 30S subunit. Two nucleotides in helix 44, A1492 and A1493, are labeled, as are rings i–iv of paromomycin and rings I–IV of hygromycin B (from Brodersen et al. [4] with copyright permission).



Ribosomal Protein Synthesis Inhibitors. Figure 3 The chemical structure of tetracycline and possible interactions with 16S rRNA in the primary binding site. Arrows with numbers indicate distances (in Å) between functional groups. There are no interactions observed between the upper portion of the molecule and 16S rRNA, consistent with data that these positions can be modified without affecting inhibitory action (from Brodersen et al. [4] with copyright permission).

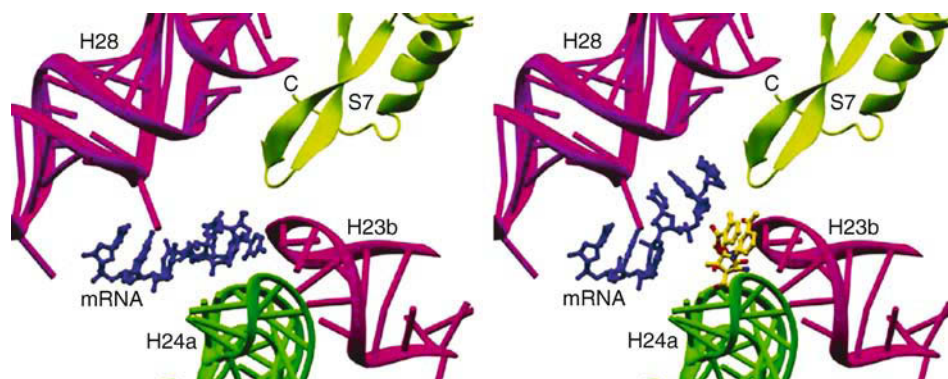
two distal aromatic rings stack on each other and G693 (Fig. 4). The central ring of the drug interacts with nucleotides in helices 23b and 24a. The stacked aromatic rings lie in the position occupied by the last two bases of the E-site codon, displacing mRNA from its normal position and disrupting interactions with the E-site tRNA (Fig. 4). The position of the bound drug also suggests that it would disrupt the ►Shine-Dalgarno interaction, which is important for initiation in bacteria.

Antibiotics and the Peptidyl Transferase Center

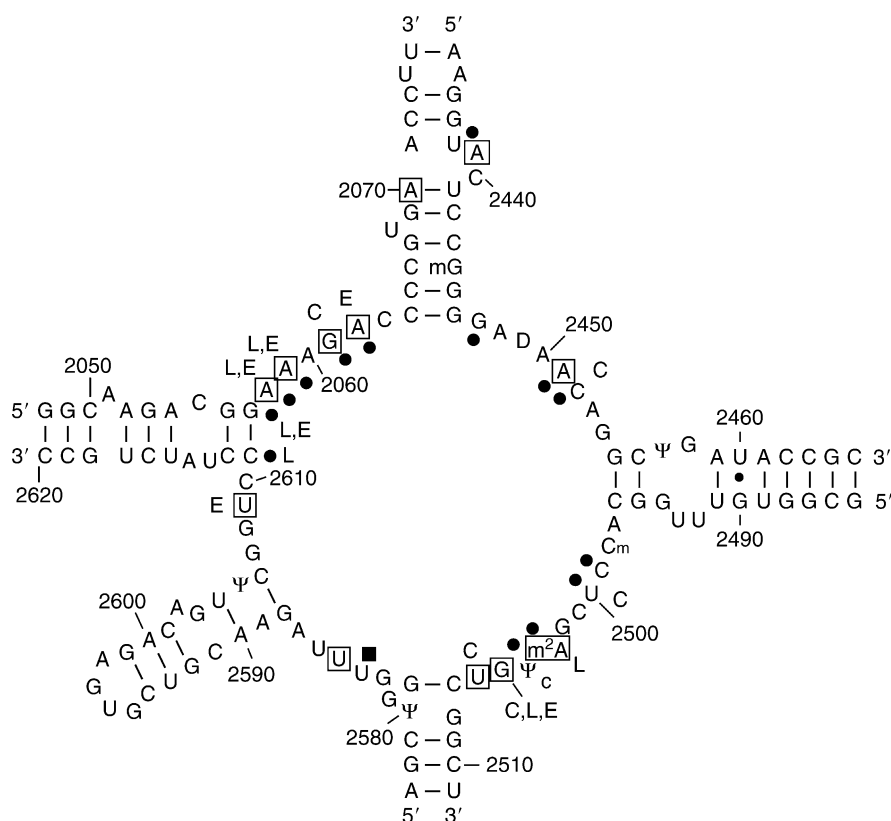
The peptidyl transferase center is the site where peptide substrates, bond formation occurs, and is located in a cavity on the interface side of the 50S subunit that leads into the peptide exit channel. It is the binding site for the 3'-termini of both donor and acceptor tRNA substrates, and is targeted by a group of structurally diverse antibiotics that either inhibit peptide bond formation directly (including ampicillin, chloramphenicol, puromycin and sparsomycin) or indirectly by interfering with movement of the nascent peptide (including erythromycin and the streptogramin B drugs). The structures of bacterial 50S-antibiotic complexes have localized binding sites of chloramphenicol, erythromycin and clindamycin that are consistent with biochemical and genetic data (Fig. 5) and represent progress towards understanding their inhibitory mechanisms [5].

Chloramphenicol is a competitor of puromycin and thereby considered to be an inhibitor of the A-site. Several functional groups on the drug are within hydrogen-bonding distance of nucleotides in the peptidyl transferase cavity including G2061, C2452, U2504, G2505 and U2506 (Fig. 5). Two divalent magnesium ions are involved in chloramphenicol binding and mediate some interactions between the drug and the peptidyl transferase cavity. Chloramphenicol binds in the A-site and may interfere with the positioning of the aminoacyl moiety and formation of the transition state.

Erythromycin is a macrolide antibiotic composed of a 14-membered lactone ring, substituted with desosamine and cladinose sugars, which does not block peptide bond formation directly. The ►macrolides are believed to function primarily by blocking the nascent peptide from the exit tunnel. Reactive groups of the desosamine sugar and the lactone ring mediate all hydrogen bond interactions with the peptidyl transferase cavity. The second generation derivatives, clarithromycin and roxithromycin, bind to the ribosome in the same fashion as erythromycin. The 2'-hydroxyl group of the desosamine sugar interacts with the N6 and N1 atoms of A2058, providing an explanation for why A2058 is essential for macrolide binding. Two common macrolide resistance mechanisms include (i) dimethylation of N6 of A2058, and (ii) rRNA mutations at



Ribosomal Protein Synthesis Inhibitors. Figure 4 The binding site of pactamycin on the 30S subunit. The positions of mRNA, the RNA elements H28, H23b, H24a, and the C-terminus of protein S7 are depicted in the E-site of the native 30S structure (*left*) and in the 30S-pactamycin complex (*right*). In the complex with pactamycin, the position of mRNA is altered (from Brodersen et al. [4] with copyright permission).



Ribosomal Protein Synthesis Inhibitors. Figure 5 Nucleotides at the binding sites of chloramphenicol, erythromycin and clindamycin at the peptidyl transferase center. The nucleotides that are within 4.4 Å of the antibiotics chloramphenicol, erythromycin and clindamycin in 50S-antibiotic complexes are indicated with the letters C, E, and L, respectively, on the secondary structure of the peptidyl transferase loop region of 23S rRNA (the sequence shown is that of *E. coli*). The sites of drug resistance in one or more peptidyl transferase antibiotics due to base changes (*solid circles*) and lack of modification (*solid square*) are indicated. Nucleotides that display altered chemical reactivity in the presence of one or more peptidyl transferase antibiotics are *boxed*.

A2058. Dimethylation causes steric hindrance and prevents hydrogen bonds to the desosamine sugar. The importance of A2058 for macrolide binding explains the selectivity of macrolides for bacterial ribosomes. Position 2058 is one of a few nucleotides in the peptidyl transferase loop that is not conserved in all phylogenetic domains, with the mitochondrial and cytoplasmic 23S rRNAs of larger eukaryotes having a G at this position. Binding of erythromycin and an adjacent magnesium ion reduces the diameter of the peptide tunnel entrance by over 50%, suggesting that macrolides can sterically block progression of the nascent peptide into the tunnel.

Clindamycin, a lincosamide antibiotic, binds to the peptidyl transferase center so as to contact both A- and P-sites. The three hydroxyl groups and carbonyl group on the drug are positioned to interact with nucleotides A2058, A2059, A2503 and G2505 (Fig. 5). Two hydroxyl groups interact with the N6 group of A2058, explaining its importance in lincosamide binding and why modifications or mutations at this position lead to drug resistance. The binding site of clindamycin suggests that it can interfere with the positioning of the 3'-ends of tRNA in the A- and P-sites, while also blocking access to the peptide tunnel.

- ▶ β -Lactam Antibiotics
- ▶ Microbial Resistance to Drugs
- ▶ Quinolones

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Ribosome-targeting Antibiotics

- ▶ Ribosomal Protein Synthesis Inhibitors

Ribozymes

Ribozymes are small RNA molecules with endoribonuclease activity. Under appropriate conditions, ribozymes exhibit sequence-specific cleavage of the target. The cleaved mRNA is destabilized and subject to intracellular degradation.

- ▶ Antisense Oligonucleotides

Rigidity

Rigidity is muscular stiffness throughout the range of passive movement in a limb segment. Cogwheel rigidity, which is typical for parkinsonism, means that tremor is superimposed on muscle stiffness.

- ▶ Parkinson's Disease
- ▶ Anti-Parkinson Drugs

RING

(really interesting new gene) finger domain: A Zn^{2+} coordinating domain found in many ubiquitin as well as SUMO E3 ligase.

- ▶ SUMOylation

RNA Editing

RNA-editing is a posttranscriptional mechanism mediated by RNA editases, which results in a site-selective deamination of adenosine to inosine. This alters codons and splicing in nuclear transcripts and thereby alters the structure and function of proteins.

- ▶ Ionotropic Glutamate Receptors

RNA Interference (RNAi) – siRNA

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Synonyms

RNA interference

Definition

RNA interference (RNAi) is triggered by double-stranded RNA (dsRNA) and causes sequence-specific mRNA degradation of single-stranded target RNAs. The mediators of mRNA degradation are small interfering RNA duplexes (▶siRNAs), which are produced from long dsRNA by enzymatic cleavage in the cell, being incorporated into a protein complex, dissociated, and used as a guiding sequence to recognize homologous mRNA that is subsequently cleaved. siRNAs are approximately 21-nucleotide length and have a base-paired structure with 2-nucleotide 3'-overhangs. Due to the catalytic nature of RNAi one siRNA can be used over and over to guide the cleavage of many mRNA molecules. Beyond their value for target validation, antisense molecules and siRNAs also hold great potential for all kinds of modulating gene expression, and furthermore as gene-specific therapeutic tools. Therefore synthetic oligonucleotides have entered clinical trials for treatment of viral diseases, cancer, and inflammatory diseases.

Description

Introduction of double-stranded RNA (dsRNA) into cells leads to the sequence-specific destruction of endogenous RNA that matches the dsRNA. The remarkable potency of the RNAi reaction enables a complete “knock-down” of a specific protein. The key enzyme required for processing of long dsRNAs to siRNA duplexes is the RNase III enzyme ▶dicer. The silencing effect is long-lasting, typically several days, and extraordinarily specific, because one nucleotide mismatch between target and the central region of the siRNA is frequently sufficient to prevent silencing. A schematic illustration of the mechanism of RNAi is shown in Fig. 1.

siRNAs can be rapidly chemically synthesized and are now broadly available. More recently, it has also become possible to express siRNAs from short inverted repeat genes in order to silence genes expressed in somatic cells. The RNAs were expressed under the control of a compact ▶RNA polymerase III promoter, which normally drives the expression of human H1 RNA, the RNA subunit of RNase P enzyme. The short hairpin loop constructs gave

rise to siRNAs presumably because ▶dicer RNase III recognizes these hairpin RNAs and excises siRNAs. Transfection of plasmids encoding siRNAs therefore represents an alternative to direct siRNA transfection and may facilitate certain applications of gene targeting. A prerequisite for the application of siRNAs for validation and therapeutic applications is the need for functional RNAi machinery within the targeted cells or tissue to bind to siRNAs and mediate mRNA degradation.

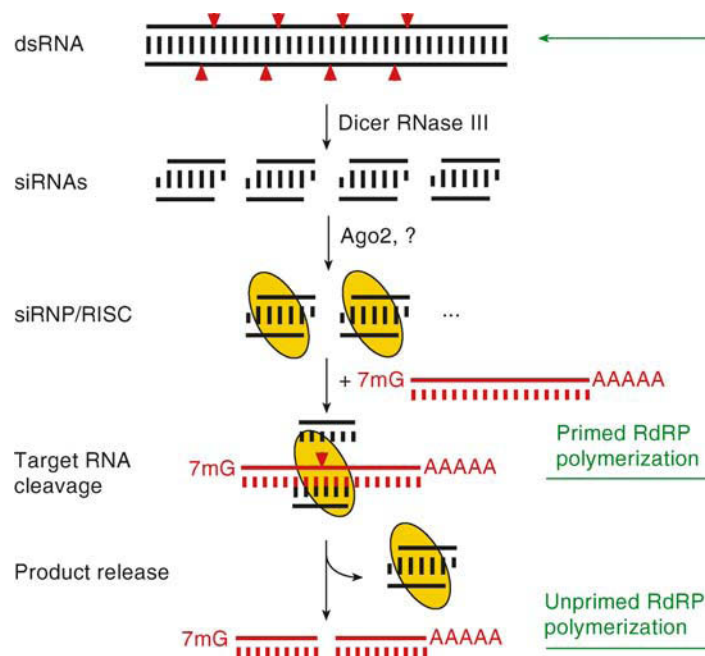
siRNAs are highly sequence-specific reagents and discriminate between single mismatched target RNA sequences and open new avenues for gene therapy. mRNAs coding for mutated proteins, which give rise to dominant genetic disorders and neoplastic growth, may be down-regulated by specific siRNAs.

Although RNAi generally works in a highly specific manner, recent studies revealed unforeseen off-target gene regulation by siRNAs. So far the small molecules have been considered to circumvent the interferon response of cells triggered by double-stranded RNA, but Sledz and co-workers demonstrated a PKR-mediated activation of the Jak–Stat pathway and a global up-regulation of interferon-stimulated genes after transfection of siRNAs. Jackson et al. used gene expression profiling to characterize the specificity of gene silencing by RNAi but found siRNA-specific rather than target-specific signatures. The signatures showed temporally distinct groups of which the fastest responders shared sequence similarities with the target site. Apart from that off-target gene silencing was caused by the sense as well as the antisense siRNA strand. Also a siRNA targeting luciferase, regulated the expression of several genes and even by decreasing the siRNA concentrations enormously the authors were unable to avoid off-target effects completely.

In sum the concept of an ideal siRNA identification attempt can be summarized as follows: (i) screen of an siRNA library, (ii) validation of the siRNA-effect by additional siRNAs targeting the same gene product, (iii) identification of the physiological role of the gene product identified, (iv) recapitulation of the in vitro findings in vivo, (v) optimization of the delivery rate to the target cells, e.g., by RNA-modifications like cholesterol-conjugation, and (vi) avoiding of unspecific and undesired siRNA effects in vivo.

Pharmacological Relevance

Especially in leukemias and lymphomas the oncogene activation frequently occurs through reciprocal ▶chromosomal translocations. These translocations split genes on both partner chromosomes leading to juxtaposition of part of each gene in the joint segments and the creation of a composite gene. Silencing of these tumor-specific chimerical mRNAs by siRNAs or ASO may become fusion gene-specific tumor therapy (Fig. 2). The extraordinary sequence specificity of the RNAi



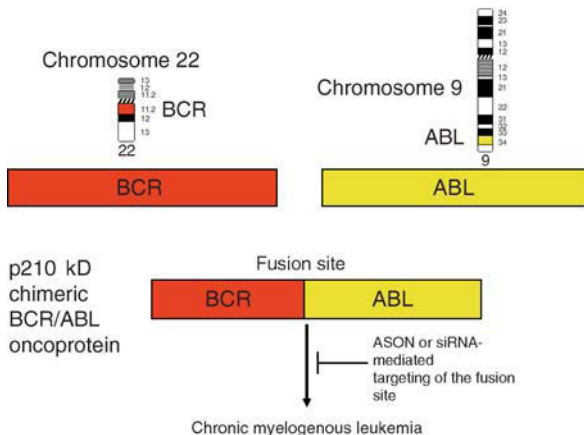
RNA Interference (RNAi) – siRNA. Figure 1 A model for RNA interference. dsRNA is processed to 21–23-nt siRNA duplexes by Dicer RNase III and possible other dsRNA-binding factors. The siRNA duplexes are incorporated into the RISC endonuclease (RNAi inducing silencing complex), which targets homologous mRNAs for degradation. Ago2 and yet to be characterized proteins are believed to be required for RISC formation. The RISC complex mediates sequence-specific target RNA degradation. In plants and nematodes, it is thought that targeted RNAs may also function as templates for double-strand RNA synthesis giving rise to transitive RNAi. Two possibilities have been suggested, siRNA-primed dsRNA synthesis or unprimed synthesis from aberrant RNA, which could represent the cleaved target RNA. In mammals or in fruit fly, however, RdRP (RNA dependent RNA polymerase) genes have yet not been identified and the major mechanism of siRNA action is believed to be endonucleolytic target RNA cleavage guided by siRNA-protein complexes (RISC).

mechanism may also allow the targeting of individual polymorphic alleles expressed in loss-of-heterozygosity tumor cells as well as point-mutated transcripts of transforming ►**oncogenes** such as RAS. The great potential of RNAi-mediated tumor therapy was first demonstrated by Brummelkamp et al. who used a retroviral version of their plasmid vector system “pSUPER” (suppression of endogenous RNA) for targeting the mutated RAS oncogene. They strongly inhibited the expression of mutated K-ras^{V12} while leaving other RAS isoforms unaffected. This extraordinary sequence specificity of RNAi, which clearly exceeds that of DNA antisense approaches, makes it a very attractive tool for cancer therapy. Brummelkamp et al demonstrated the power of RNAi-mediated gene therapy not only in cell culture but, encouragingly, in an animal model as well.

For patients with leukemia, the transfer of siRNA-based strategies into clinical applicability will certainly be both frustrating and time-consuming and many hurdles still have to be overcome in order to realize the therapeutic potential of siRNAs. Efficient delivery of siRNA into the leukemic stem cell and their unknown influence of the patient’s immune system are the most challenging issues that need to be addressed.

With respect to future medical applications, ►**siRNA** were recently directed against a pathological transcript associated with the spinal and bulbar muscular atrophy (SBMA) in tissue culture. SBMA belongs, together with the Huntington’s disease (HD), to a growing group of neurodegenerative disorders caused by expansion of trinucleotide repeats. Caplen et al. successfully targeted pathologic RNA of the androgen receptor in human kidney 293T cells that was introduced by transfection of a plasmid encoding the expanded CAG construct. Therapeutically most important, the authors achieved a rescue of the polyglutamine-induced cytotoxicity in cells treated with dsRNA molecules. Even if this study did not knockdown an endogenous disease-related transcript, the approach underlines the remarkable broad potency and sequence-specificity of RNAi mediated gene therapy. Whether the RNAi pathway is functionally active in various neuronal cells irrespective of their state of differentiation remains to be shown.

In Huntington’s disease the polyglutamine repeat expansion in exon 1 of HD leads to a toxic gain of the protein huntingtin (htt). Harper et al. could show that siRNA directed against mutant human htt reduced htt mRNA and protein expression in cell culture and in HD



RNA Interference (RNAi) – siRNA. Figure 2 A model for RNA interference. dsRNA is processed to 21–23-nt siRNA duplexes by Dicer RNase III and possible other dsRNA-binding factors. The siRNA duplexes are incorporated into the RISC endonuclease (RNAi inducing silencing complex), which targets homologous mRNAs for degradation. Ago2 and yet to be characterized proteins are believed to be required for RISC formation. The RISC complex mediates sequence-specific target RNA degradation. In plants and nematodes, it is thought that targeted RNAs may also function as templates for double-strand RNA synthesis giving rise to transitive RNAi. Two possibilities have been suggested, siRNA-primed dsRNA synthesis or unprimed synthesis from aberrant RNA, which could represent the cleaved target RNA. In mammals or in fruit fly, however, RdRP (RNA dependent RNA polymerase) genes have yet not been identified and the major mechanism of siRNA action is believed to be endonucleolytic target RNA cleavage guided by siRNA-protein complexes (RISC).

mouse brain. htt silencing improved behavioral and neuropathological abnormalities associated with HD [1]. Targeting the CAG expanded mRNA transcript by dsRNA is becoming an attractive alternative to commonly used therapeutic strategies that, beyond symptomatic treatment, mainly focus on the inhibition of the toxic effects of the polyglutaminated protein.

With respect to targeting viral gene products expressed in virus-infected cells, it should be considered that infectious mammalian viruses may express inhibitors of RNAi similar to plant viruses.

HSV2 (herpes simplex virus 2), which causes significant morbidity and is an important cofactor for the transmission of HIV infection was recently targeted in a mouse model by local application of siRNA mixed with lipids. The results suggested that siRNA could work as active components of microbicides to prevent viral infection or transmission [2].

As the repertoire of potential HIV targets is expanding, multitargeting RNAi approaches advance to inhibit HIV replication. Herein included are methods

to avoid toxicities associated with saturation of RNAi pathways or the immune stimulatory pathways by RNAi therapeutics.

A triple combination is about to enter human clinical trials for AIDS/lymphoma patients using autologous hematopoietic progenitor cells as target for vector insertion. This triple combination was introduced by Scherer et al. and consists of a lentiviral construct with three different expression cassettes (combining a U6-driven TAR RNA decoy appemdem upon a U16 snoRNA, a U6 promoted shRNA targeted to tet and rev open reading frames, and an anit-CCR5 trans-cleaving hammerhead ribozyme) [3].

siRNAs also offered protection against respiratory syncytial virus, parainfluenza and influenza. siRNA against RSV (respiratory syncytial virus, a negative strand RNA virus causing childhood respiratory diseases) has entered Phase I clinical trials in humans, and preliminary reports are promising. If appropriately formulated for improved specificity, delivery, and pharmacokinetics, siRNAs may indeed become effective antivirals in the clinics of the future.

Another field where RNAi became beneficial is the relatively isolated tissue compartment of the eye. Here RNAi has been used to identify genes that promote apoptosis or oxidative damage in retinal cells in vitro and in vivo and could therefore lead to new treatments for several ocular diseases. The feasibility of using siRNA for treatment of choroidal neovascularization using siRNA against VEGF receptor 1 or against vascular endothelial growth factor (VEGF) is being tested in clinical trials [4].

In sum, RNAi clearly has the potential to change the nucleic-based therapies for cancer, infectious diseases, and many other diseases. However, the universality of this approach, the types of genes that can be silenced using these strategies in human cells, remain unknown to date.

Outlook

MicroRNAs (miRNAs) are small (22–25 nucleotides in length) abundant class of noncoding RNAs that are believed to be important in many biological processes through regulation of gene expression by effectively reducing the translation of mRNAs binding to their 3' untranslated region (UTR). This occurs through the assembly of an RNA-induced silencing complex composed of a variety of proteins including Argonaute. If the homology between the miRNA and the target 3'-UTR is incomplete, this complex reduces expression by blocking translation. If the homology is complete, then degradation of the target mRNA can result. The precise function of miRNAs in mammals is largely unknown, but Tuschel et al. already developed a pharmacological approach for silencing miRNAs in vivo – chemically modified, cholesterol-conjugated

single-stranded RNA analogues complementary to miRNA, termed “antagomirs.” These are efficient and specific silencers of endogenous miRNAs in mice [5]. The mechanisms by which miRNA can activate or decrease gene expression is currently unknown, but may be the result of a direct (chromatin remodeling) or an indirect (suppression of transcriptional repressor) effect. Because it has been shown that miRNAs are involved in viral infection, cell growth and differentiation, insulin secretion and cancer, silencing of miRNAs with antagomirs could become a strategy for diseases.

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RNA Polymerase

Definition

The multiprotein unit that synthesizes RNA by copying the sequence information from the leading strand of the DNA. Its activity is tightly controlled by phosphorylation of the C-terminal domain (CTD), access to DNA and interaction by general and sequence specific transcription factors and coactivators and corepressors.

- Transcriptional Regulation
- Glucocorticoids

RNA Polymerase III Promoter

Promoters expressing transfer RNAs (tRNAs), U6 small nuclear (sn) RNAs, and adenovirus (Ad) virus associated (VA) RNAs are transcribed by RNA

polymerase III. They produce small, compact RNA that possess stability in the intracellular environment.

- Chromosomal Translocations
- RNA Interference (RNAi) – siRNA

RNA Polymerase Inhibitors

The inhibitors of RNA polymerase, which generates RNA from DNA, inhibit a crucial step in gene expression. Inhibition of the eukaryotic form of RNA polymerase is used in cancer chemotherapy and is also an important experimental tool. For example, actinomycin D binds to the guanine residues in DNA and blocks the movement of the eukaryotic RNA polymerase. Specific inhibitors of bacterial RNA polymerase can be used as antibacterial agents. Most of these inhibitors like rifamycin bind to the prokaryotic enzyme.

RNAi

- RNA Interference

RNAases

RNAse is an enzyme that catalyzes the breakdown of RNA molecules into their component nucleotides. RNAases are extremely common in the modern world, resulting in very short life spans for any RNA that is not in a protected environment.

- Antisense Oligonucleotides

RTK

- Receptor Tyrosine Kinase

RT-PCR

This acronym stands for Reverse Transcriptase Polymerase Chain Reaction, a method used to first copy a strand of RNA into cDNA, then amplify it through standard PCR methods.

► Gene Expression Analysis

RXR

► Retinoid X Receptor

Ryanodine Receptor

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Synonyms

Ca²⁺-induced Ca²⁺ release (CICR) channel, Foot

Definition

Ryanodine receptor (RyR) is an intracellular Ca²⁺ release channel in the ► sarcoplasmic reticulum (SR) or the endoplasmic reticulum (ER). RyR binds ryanodine (a plant alkaloid, see Drugs) with a high affinity, after which it is named.

Basic Characteristics

Occurrence and Role

There are three genetically distinct isoforms of RyR in mammals: RyR1, RyR2 and RyR3 (Table 1). RyR1 is predominantly expressed in the skeletal muscles, and RyR2 is the major isoform in the heart and, albeit in a lesser amount, the brain. RyR3 is ubiquitously expressed in a minute amount over various tissues. RyR1 and RyR2 play a pivotal role in ► excitation–contraction coupling (E–C coupling) of skeletal and cardiac muscles, respectively [1–3].

Structure

RyR forms a large homotetrameric channel composed of four identical monomers of ~5,000 amino acid

residues. Three-dimensional architectures obtained by cryo-electron microscopy demonstrates a large, fourfold symmetric cytoplasmic assembly and a small transmembrane assembly forming an ion channel (Fig. 1). The cytoplasmic assembly, which is identified as “foot” in the electron microscopic observation of the skeletal muscle triads (Fig. 3), consists of a series of interconnected tubular structures, forming such complex structure with several cavities. All the three isoforms show very similar structures. RyR forms multi-protein complex with several associated proteins, such as calmodulin (CaM) and 12 kDa FK506-binding protein ► (FKBP12) (Fig. 1c).

Mapping of functional domains in the RyR monomer is shown in Fig. 2. Although 65% amino acid residues are identical between any two of the RyR isoforms, there are three major regions of diversity, designated DR1, DR2 and DR3. DR2 is characteristically missing in RyR3. These divergent regions likely account for functional differences between these isoforms. A C-terminal ~1/10 of the sequence is hydrophobic and has 4–8 transmembrane segments that constitute the ion channel. Like many other ion channels, the two C-terminal transmembrane segments constitute part of the ion-conducting pore, and a loop between them forms a “P-loop” containing a short pore helix and the selective filter. RyR constitutes a cation channel characteristic of a large conductance (~700 pS with Na⁺ or K⁺ and ~100 pS with Ca²⁺). The RyR channel is selective for Ca²⁺ over monovalent cations (pCa/pNa = 6–7). The selectivity, however, is less than that of the voltage-dependent Ca²⁺ channel (VDCC) showing pCa/pNa > 1,000.

A large hydrophilic N-terminal region (~4,500 residues) forms the cytoplasmic assembly and serve as the regulatory domain for a large number of endogenous ligands, drugs and associated proteins (see below). The putative binding sites for FKBP12 and CaM are mapped near the middle and end of the cytoplasmic assembly, respectively.

Ca²⁺ Release Mechanisms

All the RyR channels are gated by cytoplasmic Ca²⁺, known as Ca²⁺-induced Ca²⁺ release (CICR). CICR functions as an amplifier of small Ca²⁺ signals in various excitable and non-excitable tissues and well documented in E–C coupling in the heart. In addition, RyR1 can also mediate “depolarization-induced Ca²⁺ release (DICR)”, which is controlled by some protein–protein interactions. “DICR” is the principal Ca²⁺ release mode in E–C coupling in the skeletal muscle.

Ca²⁺-Induced Ca²⁺ Release and its Modulators

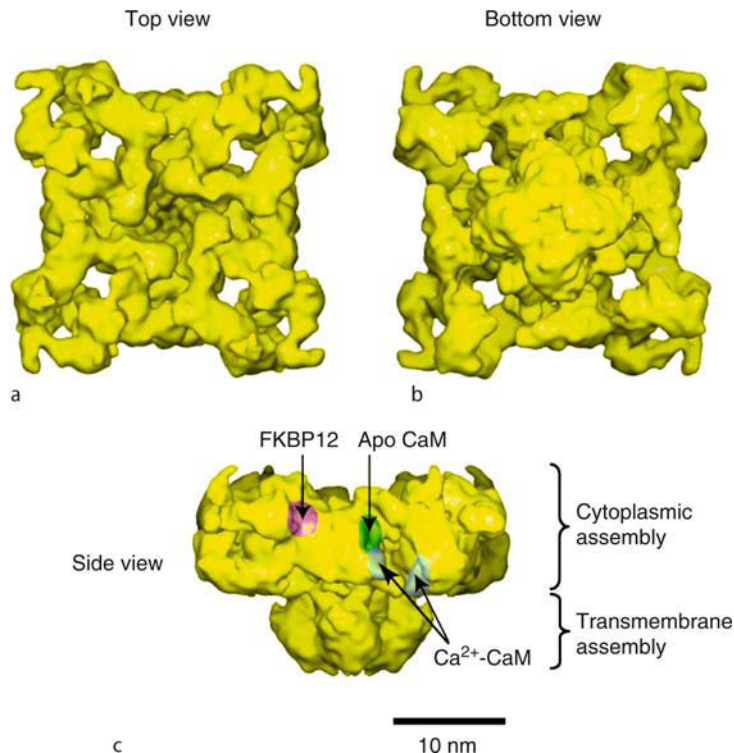
In CICR, the RyR channel is activated by μM Ca²⁺ and inhibited by mM Ca²⁺. This biphasic Ca²⁺ dependence can be explained by two distinct Ca²⁺ binding sites: high-affinity (K_D ~ μM) Ca²⁺ site for activation (A-site)

Ryanodine Receptor. Table 1 Three Mammalian RyR isoforms

	RyR1	RyR2	RyR3
Gene locus (human)	19q13.1	1q42.1–43	15q14–15
Amino acid residues	5,037	4,976	4,872
<i>Occurrence</i>			
Peripheral	Skeletal muscle	Heart	Ubiquitous (minuscule)
			Skeletal muscle ^a
CNS	Purkinje cells	Ubiquitous	Hippocampus
			Corpus striatum
<i>Trigger for Ca²⁺ release</i>			
Ca ²⁺ (CICR)	Yes	Yes	Yes
Ca _v 1.1 (DICR)	Yes	No	No
Knockout mice	Perinatally lethal	Embryonic lethal	No gross abnormalities ^b
Related diseases	MH/CCD/MmD	CPVT/ARVD	Unknown

^aRyR3 is temporarily expressed in mammalian skeletal muscle during development and down-regulated to a negligible amount in adult except for diaphragm and soleus muscles. In some nonmammalian vertebrates (e.g. fish, frogs and birds), nearly equivalent amounts of RyR1 and RyR3 are expressed in adult skeletal muscles.

^bSome alterations in CNS function (e.g. enhanced locomotor activity or alterations in synaptic plasticity) are reported.

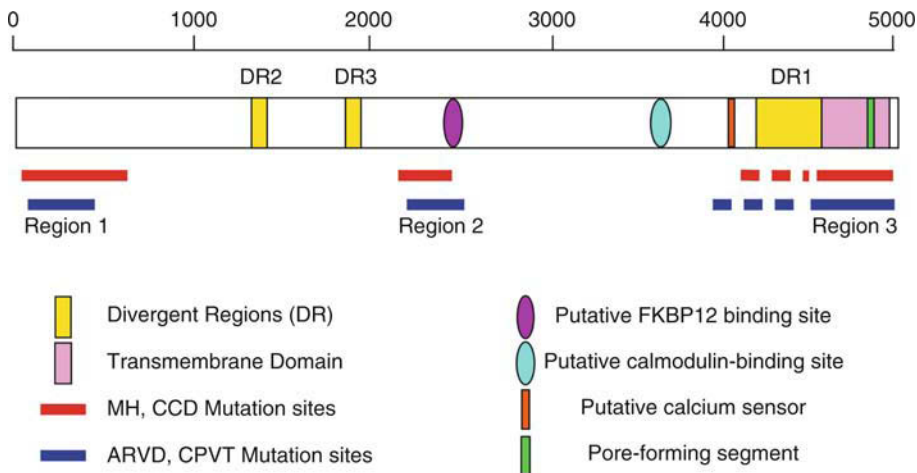


Ryanodine Receptor. Figure 1 Three-dimensional architecture of the RyR1 by cryo-electron microscopy. (a), top view (from the T-tubule); (b), bottom view (from the SR lumen); (c), side view (parallel to the SR membrane). The binding sites of FKBP12, apo-CaM and Ca²⁺-CaM are indicated in the side view. Courtesy of Dr. M. Samso (modified from Samso et al. (2005) Nat Struct Mol Biol 12: 539–544).

and low-affinity ($K_D \sim \text{sub-mM}$ (RyR1) to $\sim \text{mM}$ (RyR2)) Ca²⁺ site for inactivation (I-site). In addition to the occupation with Ca²⁺ of the A- and I-sites, the CICR activity of RyR is also determined by a third

parameter independent of Ca²⁺ which sets the maximal attainable level.

CICR is modulated by several endogenous ligands. Mg²⁺ inhibits CICR by acting as a competitive



Ryanodine Receptor. Figure 2 Schematic illustration of characteristic structure in the linear sequence of the RyR monomer. Structural features of the RyR monomer are depicted with RyR1 as the representative of the three isoforms. A C-terminal ~500 residues (pink) form hydrophobic transmembrane assembly that constitute the ion channel. Pore-forming segment (P-loop) is marked in green. DR1, DR2 and DR3 represent divergent regions between the isoforms. DR2 is characteristically missing in RyR3. The putative binding sites for FKBP12 and CaM are indicated in purple and light blue, respectively. The putative Ca^{2+} sensor is in orange. Mutated sites linked to malignant hyperthermia (MH) and central core disease (CCD) cluster in the three regions (regions 1–3) of the *RyR1* gene (red bars). Mutation sites in RyR2 associated with CPVT and ARVD also cluster in similar regions (blue bars).

antagonist to Ca^{2+} in the A-site and as an agonist of Ca^{2+} in the I-site. In addition to these effects, a cytoplasmic concentration (0.3–1 mM) of Mg^{2+} is necessary for RyR2 to function properly. Adenine nucleotides such as ATP greatly stimulate CICR without changing Ca^{2+} sensitivity. Oxidation or *N*-nitrosylation of some hyperreactive sulfhydryl residues stimulates CICR activity. Cytoplasmic pH affects CICR: acidification decreases the activity and alkalization increases it. The effect of phosphorylation on RyR is controversial. The CICR activity may be modulated by a large number of associated proteins. Among them, CaM and FKBP12 are well investigated. CaM binds RyR on the cytoplasmic side (Figs. 1 and 2). The action of CaM is isoform-dependent: it activates RyR1 and RyR3 at a low (submicromolar) Ca^{2+} and inhibits at a higher Ca^{2+} , whereas it inhibits RyR2 irrespective of Ca^{2+} concentrations. FKBP12 similarly interacts with RyR1 to stabilize the channel activity (Figs. 1 and 2).

The RyR channels seem to be regulated by luminal Ca^{2+} . Luminal Ca^{2+} may activate the RyR2 channels in the heart. The association of **calsequestrin** with RyR2 via triadin or junctin is proposed as a possible regulatory mechanism. Such activation by luminal Ca^{2+} remains controversial in the skeletal muscle.

E-C Coupling in Heart

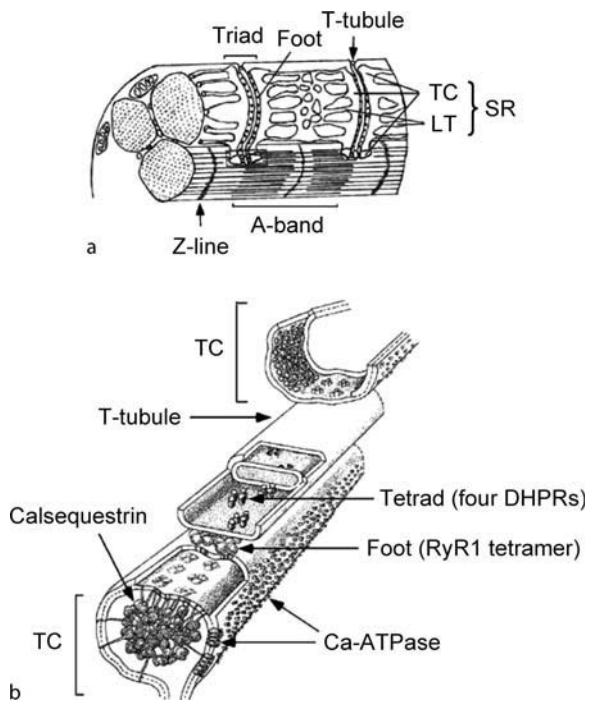
In E–C coupling in the heart, the RyR2 channel releases Ca^{2+} from the SR on depolarization of the plasma membrane or T-tubules by CICR mechanisms. The

cardiac junctional SR form dyad structure (similar to triad structure in the skeletal muscle, see below) with the plasma membrane or T-tubule, where L-type VDCC (cardiac **dihydropyridine receptor** (DHPR) containing $\text{Ca}_v1.2$ as the pore-forming subunit) is closely apposed to RyR2 in the SR. RyR2 is activated by Ca^{2+} , which enters via the DHPR on depolarization. Thus, cardiac muscle contraction requires extracellular Ca^{2+} . “ **Ca^{2+} sparks**” are thought to be an elementary event of the Ca^{2+} release in which multiple RyR2 channels in a cluster open synchronously. In the Ca^{2+} overloaded muscles, Ca^{2+} release via RyR2 may occur spontaneously without Ca^{2+} influx, and propagate in the cell as **Ca^{2+} waves**.

Similar Ca^{2+} release mechanism operates also in smooth muscles, neurons and some peripheral tissues.

E-C Coupling in Skeletal Muscle

In E–C coupling in vertebrate skeletal muscle, the RyR1 channel releases Ca^{2+} from the SR on depolarization of the T-tubules. This process seems similar to that in the heart, but skeletal muscle contraction does *not* require Ca^{2+} influx that triggers CICR. The mechanism is known as “depolarization-induced Ca^{2+} release (DICR)”. DICR requires regular and specific configuration of molecules involved. In skeletal muscle, T-tubule invaginates at the specific sites to form “triad” structure with the terminal cisternae (TC) of the SR on both sides (Fig. 3a). At the triad, four skeletal muscle-type DHPRs (containing $\text{Ca}_v1.1$) assemble into “tetrad” and are closely apposed (coupled) in precise register to



Ryanodine Receptor. Figure 3 Schematic drawings of mammalian skeletal muscle (a) and characteristic configuration of the components essential for E–C coupling in the triad (b). (a), the T-tubule invaginates at the A–I junction to form triad structure with terminal cisternae (TC) of the SR. LT, longitudinal tubule of the SR. Modified from Fawcett (1994) Bloom and Fawcett a Textbook of Histology (12th ed., Chapman & Hall, New York and London), p278. (b), at the triad, two rows of feet (RyR1 tetramers) are aligned in the junctional face of the TC, and tetrads (four DHPRs) in T-tubules are located in precise register with opposite alternate feet. Modified from Block et al (1988) J Cell Biol 107: 2587–2600.

the alternate RyR1 homotetramers (feet), which are aligned in two rows on each junctional face of the TC (Fig. 3b). The current view of DICR is that depolarization of T-tubule induces the conformation change in the $\text{Ca}_v1.1$, which in turn, activates the coupled RyR1 channels directly or indirectly (via other proteins such as the $\beta 1$ subunit of DHPR). In this view, DHPR serves as the voltage sensor rather than the Ca^{2+} channel. Thus, DICR is thought to occur on the basis of the $\text{Ca}_v1.1$ -RyR1 interaction. The RyR1- $\text{Ca}_v1.1$ interaction is specific and cannot be replaced by any other isoforms of RyR or DHPR. Studies with chimeric RyRs have proposed that multiple regions within the cytoplasmic assembly may be involved in this interaction.

In the skeletal muscle, RyR1 can also mediate CICR. Therefore, it may reasonably be hypothesized that Ca^{2+} released from the RyR coupled with DHPR might

induce CICR via the uncoupled RyR in physiological **twitch** or **tetanus**. The rate of CICR estimated under physiological condition, however, is much slower than the rate of DICR, suggesting only minor contribution of CICR to the physiological muscle **contraction**. The slow CICR rate is primarily due to a low maximal attainable level of the CICR activity of RyR1. Enhanced CICR is implicated in the pathogenesis of some muscle diseases (see Disease).

Diseases

Several human diseases are associated with mutations in RyR1 or RyR2, whereas RyR3-linked diseases are unknown so far. Mutations in RyR1 are linked to some cases of **malignant hyperthermia (MH)**, **central core disease (CCD)** and multi-minicore disease (MmD) [4]. Mutations in RyR2 are associated with **catecholaminergic polymorphic ventricular tachycardia (CPVT)** and a form of **arrhythmogenic right ventricular dysplasia (ARVD)** [5]. These mutated sites cluster in three regions: the N-terminal region (region 1), the central region (region 2), and the C-terminal region (region 3) that includes the transmembrane region (Fig. 2).

These mutations may alter the Ca^{2+} release channel functions. MH mutations in RyR1 largely cluster in the cytoplasmic regions 1 and 2 and tend to make the channel easier to open (enhanced CICR activity), whereas most CCD mutations are in the pore-forming region 3 and seem to form the “leaky channel” at resting cytoplasmic Ca^{2+} . Some CCD mutations lead to a severe inhibition of pore opening. Alterations of Ca^{2+} handling are reported in the RyR2 channels carrying CPVT and ARVD mutations.

Drugs Ryanodine

Ryanodine is a neutral plant alkaloid from *Ryania speciosa* and was used as an insecticide. It also has been well known by the characteristic action on mammalian skeletal muscle of slowly developing, and intensive and irreversible **contracture**. Ryanodine binds specifically to the “open” RyR channel at the stoichiometry of 1 mol/mol homotetramer with a high affinity ($K_D \sim \text{nM}$) and leads the channel to “ryanodine modified” state characteristic of long-lasting subconductance ($\sim 50\%$ of normal) opening. At higher concentration, it blocks the channel.

Ryanodine leads to contracture of mammalian skeletal muscle, whereas it causes negative inotropism in cardiac muscle. This apparent opposite effects are due to difference in the Ca^{2+} extruding activity: the released Ca^{2+} remains within cytoplasm in skeletal muscle because of low Ca^{2+} extruding activity, whereas the increased cytoplasmic Ca^{2+} is rapidly excluded out of the cytoplasm in cardiac muscle via Na^+ - Ca^{2+} -exchange.

Caffeine

Caffeine is a strong activator of CICR: it sensitizes RyR to activating Ca^{2+} and increases the maximum attainable level. Because high concentrations (>mM) of caffeine effectively discharge Ca^{2+} from the Ca^{2+} store, it is frequently used for experimental evaluation of functional occurrence of RyRs. Caffeine is used for diagnosis of MH: muscles biopsied from MH patients contract by a lower dose of caffeine than normal human, due to an enhanced CICR activity (see Disease)./Para>

4-Chloro-m-cresol (4-CmC)

4-CmC, a preservative in commercial preparations of some intravenous drugs, activates CICR in a way similar to that of caffeine. 4-CmC is more potent than caffeine and shows isoform-dependent activation profiles: it is much less effective in RyR3 than RyR1 or RyR2.

Inhalation General Anaesthetics (halothane)

Inhalation general anaesthetics, such as halothane, stimulate CICR in a way similar to that of caffeine. Administration of these agents to patients with MH mutations in *RyR1* gene will cause the MH episodes.

FK506 and Rapamycin

These immunosuppressants increase the activity of RyR1 by deprivation of FKBP12. Their effect on RyR2, which binds FKBP12/12.6, is controversial. RyR3 could bind FKBP12 with unchanged activity.

Dantrolene

Dantrolene is an antidote for MH, which inhibits the CICR activity. Action of dantrolene may be isoform specific (less effective on RyR2), Ca^{2+} -dependent (more effective at a lower Ca^{2+} concentration), and temperature-dependent (more effective at 37°C than at 25°C).

Procaine and Tetracaine (Local Anaesthetics)

They decrease the CICR activity without changing the Ca^{2+} sensitivity. Tetracaine is more potent than procaine. It should be noted that some local anaesthetics, e.g. dibucaine may stimulate CICR.

Ruthenium Red

Ruthenium red inhibits the CICR activity at μM range. The site of ruthenium red action seems to be near or within ion channels. It also inhibits mitochondrial function.

►IP₃ Receptors

References

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Ryk/Derailed

Ryk and its *Drosophila* homolog, derailed, are receptor tyrosine kinases which have been shown to act as co-receptors with the Fz proteins for Wnt signaling. The first evidence for an interaction with Wnts came from genetic studies in *Drosophila* which showed axon-guidance defects due to misexpression of *derailed* that was rescued by deletion of the *wnt5* gene. Later work in mice confirmed the role of Ryk in axon guidance and in Wnt signal transduction by linking similar axon defects to the presence or absence of Ryk and Wnt3 in affected neurons. In mammalian cell culture Ryk can interact with both Fz and Dvl. Wnt signaling mechanisms downstream of Ryk are not well defined, but probably do *not* rely on Ryk kinase activity, as the kinase domain has been shown to be without normal activity.

►Wnt Signaling

S6K1

S6K1 (also known as p70S6 kinase) is a serine/threonine protein kinase which is involved in the regulation of translation by phosphorylating the 40S ribosomal protein S6. Insulin and several growth factors activate the kinase by phosphorylation in a PI 3-kinase dependent and rapamycin-sensitive manner. Phosphorylation of S6 protein leads to the translation of mRNA with a characteristic 5' polypyrimidine sequence motif.

► **Insulin Receptor**

S-Prenylation

► **Lipid Modifications**

S100 Proteins

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Synonyms

Ca²⁺ regulation; S100 proteins; EF-hand; RAGE

Definition

► **S100 proteins** (named because of their solubility in a 100% ammonium sulfate solution) constitute the largest family within the EF-hand Ca²⁺-binding proteins superfamily [1]. S100 proteins are small, acidic proteins containing two distinct Ca²⁺-binding ► **EF-hand** structural motifs [2]. The C-terminal EF-hand contains the

classical Ca²⁺-binding motif, common to all EF-hand proteins. This Ca²⁺-binding motif has a typical sequence signature of 12 amino acids and is flanked by two helices. The N-terminal EF-hand, different from the classical EF-hand motif, contains a 14 amino acid consensus sequence motif, named the 'S100-specific' or 'pseudo' EF-hand.

Among the different human S100 genes, most are clustered on human chromosome 1q21, a region frequently involved in chromosomal rearrangements in cancer. Based on these findings, an official nomenclature for S100 proteins was introduced (Table 1).

► <http://www.gene.ucl.ac.uk/nomenclature/index.html>

S100 proteins have multiple biological functions in cells; some are secreted from cells and activate receptor for advanced glycation end products (RAGE)-dependent cellular pathways.

Basic Characteristics

Intracellular Ca²⁺-levels are controlled by release into, and removal from, the cytoplasm (Fig. 1). Ca²⁺-pumps in the plasma membrane and endoplasmic reticulum (ER; the Ca²⁺-store in a cell) keep cytoplasmic Ca²⁺-levels low (about 0.1 µmol/L in resting cells) and generate a 10,000-fold concentration gradient across membranes (because extracellular Ca²⁺ is in the millimolar range). Upon stimulation, Ca²⁺ enters the cytosol of the cell via Ca²⁺-channels (plasma membrane) or via Ca²⁺-channels in the ER, leading to the activation of a great variety of Ca²⁺-dependent processes in the cell.

Ca²⁺-channels mediate Ca²⁺-entry into cells and Ca²⁺-pumps of the plasma membrane and of the endoplasmic reticulum remove the ion from the cytoplasm. The Ca²⁺-signal is mediated by the EF-hand Ca²⁺-binding proteins (including the S100 protein family) which have INTRA- and EXTRACELLULAR cytokine-like functions. Ligands including S100 proteins activate RAGE of neighbouring cells leading to physiological or pathological responses. RAGE ligands: S100 proteins; AGEs: Advanced Glycation End Products amphoterin/high mobility group 1 proteins (HMG-1); LPS, (lipopolysaccharide/endotoxins) β-amyloid.

RAGE, (domains V, C1, and C2) belongs to the immunoglobulin family of proteins.

A large group of diseases is associated with mutations in Ca²⁺-channels (channelopathies) and

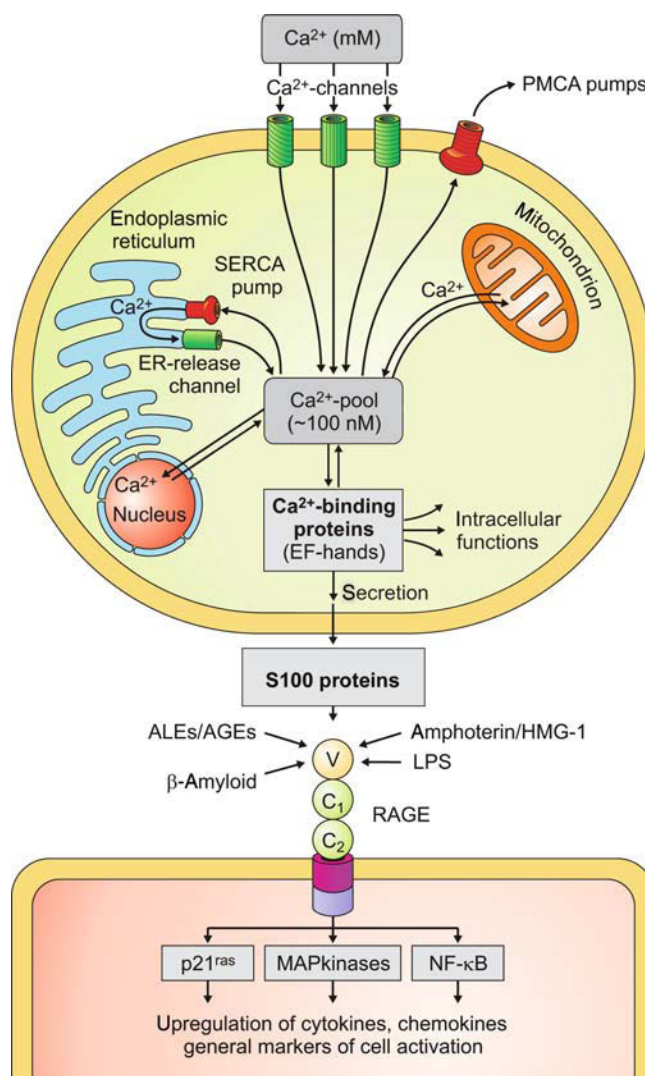
S100 Proteins. Table 1 Nomenclature^a and chromosomal location of the S100 genes

Approved gene symbol	Approved gene name	Previous symbols and aliases	Chromosomal location	Sequence accession ID
S100A1	S100 calcium binding protein A1	S100A, S100-alpha	1q21	NM_006271
S100A2	S100 calcium binding protein A2	S100L, CaN19	1q21	NM_005978
S100A3	S100 calcium binding protein A3	S100E	1q21	NM_002960
S100A4	S100 calcium binding protein A4	Calvasculin, metastasin, murine placental homolog, calcium placental protein (CAPL), MTS1, p9Ka, 18A2, pEL98, 42A	1q21	NM_002961
S100A5	S100 calcium binding protein A5	S100D	1q21	NM_002962
S100A6	S100 calcium binding protein A6	Calcyclin (CACY), 2A9, PRA, CABP	1q21	NM_014624
S100A7	S100 calcium binding protein A7	Psoriasin 1 (PSOR1), S100A7c	1q21	NM_002963
S100A7A	S100 calcium binding protein A7A	S100A15, S100A7L1	1q21	NM_176823
S100A7L2	S100 calcium binding protein A7-like 2	S100A7b	1q21	–
S100A7P1	S100 calcium binding protein A7 pseudogene 1	S100A7L3, S100A7d	1q21	–
S100A7P2	S100 calcium binding protein A7 pseudogene 2	S100A7L4, S100A7e	1q21	–
S100A8	S100 calcium binding protein A8	Calgranulin A (CAGA), CGLA, P8, MRP8, CFAG, LIAg, 60B8AG	1q21	NM_002964
S100A9	S100 calcium binding protein A9	Calgranulin B (CAGB), CGLB, P14, MRP14, CFAG, LIAg, 60B8AG	1q21	NM_002965
S100A10	S100 calcium binding protein A10	Annexin II ligand (ANX2LG), calpactin I, light polypeptide (CAL1L), p11, CLP11, 42C	1q21	NM_002966
S100A11	S100 calcium binding protein A11	Calgizzarin, S100C	1q21	NM_005620
S100A11P	S100 calcium binding protein A11 pseudo-gene	S100A14	7q22–q31	–
S100A12	S100 calcium binding protein A12	Calgranulin C (CAGC), CAAF1, CGRP, p6, EN-RAGE	1q21	NM_005621
S100A13	S100 calcium binding protein A13		1q21	NM_005979
S100A14	S100 calcium binding protein A14	BCMP84, S100A15	1q21	NM_020672
S100A16	S100 calcium binding protein A16	S100F, DT1P1A7, MGC17528	1q21	NM_080388
S100B	S100 calcium binding protein B	S100-beta	21q22	NM_006272
S100G	S100 calcium binding protein G	Calbindin 3 (CALB3), CaBP9K, CABP1	Xp22	NM_004057

S100 Proteins. Table 1 Nomenclature^a and chromosomal location of the S100 genes (Continued)

Approved gene symbol	Approved gene name	Previous symbols and aliases	Chromosomal location	Sequence accession ID
S100P	S100 calcium binding protein P		4p16	NM_005980
S100Z	S100 calcium binding protein Z	S100-zeta	5q13	NM_130772

^aMarenholz et al (2006) Biochim Biophys Acta 1763:1282–1283.

**S100 Proteins. Figure 1** Pathways of Ca^{2+} -release and uptake.

Ca^{2+} -pumps. After entering the cell, Ca^{2+} is reversibly complexed to specific Ca^{2+} -binding proteins that fulfil multiple functions, including Ca^{2+} -buffering and transport, activation of enzymes, regulation of contraction,

secretion, and proliferation. This large family of Ca^{2+} -binding proteins is characterized by a common structural element, the EF-hand [2, 3]. These proteins decipher the information carried by Ca^{2+} and pass it on

to various targets regulating biological functions. The largest group within the EF-hand Ca^{2+} -binding proteins are the S100 proteins. S100 proteins are cell- and tissue-specific expressed and regulate many intra- and extracellular processes by interacting with specific target proteins.

In general the affinities of S100 proteins for Ca^{2+} are low with a binding affinity of 100–500 μM . Binding of Ca^{2+} to S100 proteins induces helix rearrangement within each subunit of the dimer, resulting in the exposure of two hydrophobic surfaces (one in each monomer), which are involved in target protein recognition. Beside Ca^{2+} , a number of S100 proteins can also bind Zn^{2+} or Cu^{2+} that can influence the affinity of Ca^{2+} -binding. Thus, S100 proteins display variable transition metal-binding properties in agreement with their highly diversified and specialized functions, such as cell proliferation, migration differentiation, as well as in cognition.

Recent advances in the elucidation of the biological functions of a selection of S100 proteins are summarized below.

In response to a rise up calcium, S100 proteins interact with distinct target proteins and some members relocate to different cellular compartments and are implicated in multiple intracellular and extracellular activities.

The brain-specific S100B regulates enzyme activity by rendering certain phosphorylation sites inaccessible to their interaction partners, as was reported for neurogranin, tau protein, glial fibrillary acid proteins (GFAP), and the p53 transcription factor. In addition, several S100 proteins have been shown to be secreted via different mechanism, including the classic endoplasmic reticulum-Golgi pathway, or alternative pathways involving cytoskeletal components, such as actin and tubulin. S100B secretion from astroglial cells is induced by serotonin 1a receptor agonist binding. Moreover, the release of S100 protein from apoptotic cells following brain injury may be sufficient to induce cellular responses. In vitro, extracellular S100B and S100A4 promote neurite outgrowth and neuronal survival by acting through **RAGE** and heparan sulfate proteoglycans, respectively, both located at the cell surface. Moreover, extracellular S100B has been shown to modulate long-term neuronal synaptic plasticity, glutamate uptake of hippocampal astrocytes, and inflammation.

S100A1 is the most abundant in the myocardium but is also expressed in brain and other tissues. S100A1 was found to stimulate Ca^{2+} -induced Ca^{2+} -release (CICR) in skeletal muscle terminal cisternae. In the presence of nanomolar Ca^{2+} -concentrations, S100A1 binds to the ryanodine receptor increasing its channel open probability, and was shown to enhance SR Ca^{2+} -release and contractile performance. Several animal models (over expressing S100A1 or S100A1-deficient mice) have

emphasized the importance of S100A1 in calcium-cycling of the heart and its association with heart failure.

Furthermore, we also found behaviour differences in our mouse models of S100A1 deficiency. The male S100A1 knockout mice showed reduced anxiety-like responses and enhanced explorative activities and we concluded that S100A1 plays a role in modulating innate fear and exploration of novel stimuli.

S100A2 encoding cDNA was first identified as a novel tumour suppressor gene. S100A2 interacts in a Ca^{2+} -dependent manner with the tumour suppressor p53 and activates its transcriptional activity. S100A2 was shown to interact with the same p53 binding site as S100B.

A recent DNA microarray-based search for proliferation control genes showed that 28 genes are cooperatively activated by IFN- α and TGF- β . Among these are the insulin-like growth factor-binding protein 3 (IGFBP3) and S100A2. IGFBP3 protein was found to be a potent growth inhibitor requiring TGF- β activity. The antiproliferative activity of S100A2 is significantly enhanced by IFN- α in stably transfected human melanoma cell lines. IFN- α was found to be a potent inducer of intracellular Ca^{2+} -release required for activation of S100A2. This study provides a functional link between IFN- α and TGF- β signalling and extends the functions of IFN signalling to calcium-sensitive processes.

S100A4 is implicated in tumour progression and metastasis and high S100A4 expression levels have been correlated with reduced patient survival and poor prognosis. The relevance of S100A4 to clinical parameters was investigated in several archival colon cancer specimens, without metastasis at the time of surgery [4]. Remarkably, S100A4 mRNA levels were found to be higher in the primary tumours, which later developed distant metastases. Patients whose tumours were heterozygous for activating β -catenin mutation were identified, and the tumours showed both nuclear β -catenin staining and high S100A4 expression. All these patients developed metastases in the liver. This was the first demonstration that mRNA expression of S100A4, determined in primary tumours in a quantitative manner, is of value for the prediction of metastasis cancer.

The results also showed an interconnection of two previously unconnected molecular pathways which play important roles in tumour progression and metastasis, the β -catenin/TCF signalling pathway and S100A4, that controls motility and invasiveness. The finding demonstrated that β -catenin/TCF directly regulates the expression of the S100A4, and that β -catenin-induced effects on cell migration and invasion are mediated by S100A4 in colon cancer cells. New therapeutic strategies aimed at disrupting this regulation

and the function of S100A4 protein may be of particular value for prevention of colon cancer metastasis.

S100A10 was found to interact with the serotonin 1B receptor increasing its presence at the cell membrane. S100A10 was found to be closely associated with the pathophysiology of depression.

S100A16 is the latest example. This protein was found to accumulate within nucleoli of glioblastoma cells and translocate to the cytoplasm in response to Ca^{2+} -stimulation. This suggested a possible role of S100A16 in ribonucleoprotein complex processing, gene silencing, or cell cycle progression. Like other members of the family, S100A16 lacks the canonical nuclear localization signal. Nuclear import may require interaction with transporter proteins or phosphorylation, as it was described for S100A11, or may occur via facilitated diffusion pathways, as observed for calmodulin.

S100 Proteins and RAGE Signalling

Some S100 proteins are secreted from cells and exert their extracellular functions through their interaction with the [receptor for advanced glycation end products \(RAGE\)](#) [5]. RAGE is a member of the immunoglobulin-like cell surface receptor superfamily with multiple functions. RAGE is composed of three extracellular immunoglobulin domains, (a 'V' domain followed by two 'C' type domains), a single spanning transmembrane region, and a short cytosolic domain (Fig. 1). Recently, RAGE isoforms lacking the transmembrane and cytosolic regions (endogenous

secreted RAGE or esRAGE) or the 'V' immunoglobulin domain (N-truncated RAGE or NtRAGE) were identified in the human brain. RAGE can be activated by S100 proteins, amyloid- β ($\text{A}\beta$), amphotericin, LPS, or advanced glycation end products (AGEs). The RAGE ligands activate different signalling pathways, p21^{ras} and mitogen-activated protein kinases (MAPK). Depending on the pathways activated the RAGE ligand signal leads to the activation of transcription factors like NF- κB or Sp1 and to cellular responses. Activation of RAGE enhances the expression of the receptor itself and initiates a positive feedback loop, resulting in sustained RAGE up-regulation, which has been suggested to be the starting point of chronic cellular activation and tissue damage. Several studies have succeeded to block the RAGE/ligand interactions, resulting in a suppression of atherosclerosis and improved wound healing in diabetic animal models.

Drugs

Disease Association and Molecular Drug Targets

Proteins of this family are closely associated with a number of human diseases such as cardiomyopathy, inflammation, cancer, Alzheimer's disease, and depression (Table 2). They have also been proven to be valuable in the diagnosis of these diseases (Table 3) in order to improve clinical management, outcome and survival of patients. They are considered as having a potential as drug targets to improve therapies. Moreover, S100A1 gene therapy is suggested as a novel

S100 Proteins. Table 2 S100 proteins in human diseases

Proteins	Association with diseases
S100B	Developmental brain dysfunction, learning and memory deficits Alzheimer's disease, blood brain barrier dysfunction Down syndrome
S100A1	Cardiomyopathy, anxiety
S100A2	Cancer, tumour suppression
S100A4	Cancer, metastasis
S100A6	Cancer, amyotrophic lateral sclerosis (ALS)
S100A7	Psoriasis
S100A8/A9	Inflammation, cystic fibrosis Wound healing, juvenile rheumatoid arthritis
S100A10	Depression
S100A11	Cancer
S100A12	Inflammation, Kawasaki disease, Mooren's ulcer
S100P	Cancer

References: Heizmann (2005) J Pediatrics 147:731–738; Heizmann et al (2007) In: Carafoli E, Brini M (eds) Calcium signalling and disease. Springer pp. 93–138; Foell et al (2004) Arthritis & Rheumatism 50:1286–1295; Donato et al (2003) Microsc Res Tech 60:540–551; Kwon et al (2005) Front Biosci 10:300–325; Foell et al (2003) Lancet 361:1270–1272; Svenningsson et al (2006) Science 311:77–80; Ackermann et al (2006) Biochim Biophys Acta 1763:1307–1319; Foser et al (2006) Cell Mol Life Sci 63:2387–2396.

S100 Proteins. Table 3 S100 proteins in clinical diagnostics

Proteins	Diagnostic markers
S100B	Sensitive marker of hypoxic brain damage in infants and children undergoing open-heart surgery
	Pediatric traumatic brain injury
	Blood brain barrier dysfunction
	Marker for prenatal screening for trisomy 21
	CNS lesions in PKU
	Developmental brain dysfunction
	Hypoxic-ischemic encephalopathy in asphyxiated full-term infants
S100A1	Marker for acute myocardial ischemia
S100A2–S100A6	Immunohisto-chemical classification of tumours in the brain
	Tumour prognosis and clinical management
	Marker to amyotrophic lateral sclerosis (ALS)
S100A8/A9	Inflammatory disorders including rheumatoid arthritis and chronic bronchitis
S100A12	Juvenile rheumatoid arthritis
	Mooren's ulcer (autoimmune disease)
	Acute and chronic inflammatory diseases
S100A14/S100A16	Detection of circulating tumour cells in peripheral blood

References: Heizmann et al (2002) *Front Biosci* 7:d1356–d1368; Rothermundt et al (2003) *Microsc Tech Res* 60:614–632; Michetti et al (2002) *Clin Chem* 48:2097–2104; Gazzolo et al (2003) *Arch Pediatr Adolesc Med* 157:1163–1168; Foell et al (2004) *Clin Chim Acta* 344:37–51; Gazzolo et al (2004) *Crit Care Med* 32:131–136; Kanner et al (2003) *Cancer* 97:2806–2813; Schulpis et al (2004) *Clin Biochem* 37:76–79; Thorngren et al (2004) *Pediatr Res* 55:406–412; Smirnov et al (2005) *Cancer Res* 4993–4997.

treatment to improve existing contractile performance, e.g. after myocardial infarction.

Outlook

Many vital physiological functions and metabolic processes are regulated by Ca^{2+} and a large number of human diseases are linked to an altered Ca^{2+} -homeostasis. The Ca^{2+} -signal within cells is transmitted by many Ca^{2+} -binding proteins, including the S100 proteins family. S100 proteins have received increased attention recently because of their close association with human diseases and their use in diagnosis. S100 proteins are also considered (together with their extracellular receptor RAGE) to have a potential as drug targets to improve therapies in the future.

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S-Acylation

►Lipid Modifications

S-adenosyl-L-methionine

S-adenosyl-L-methionine (AdoMet, SAM) is a cofactor and the most important donor of the methyl (CH_3 -) group for methyltransferases, including COMT. When the methyl-group has been transferred, the remaining demethylated compound is called S-adenosyl-L-homocysteine.

►Catechol-O-Methyltransferase and its Inhibitors

Salicylate

►Non-steroidal Anti-inflammatory Drugs

SAM

► S-Adenosyl-L-methionine

SAR

Structure activity relationships, i.e., the total pattern of change in a biological activity as a function of chemical structure, typically derived from a comparison within a chemical series so that the biological effects of substitution at each structural position may be determined and correlated.

SARA

(Smad anchor for receptor activation) An intracellular protein Sara which accumulates at early endosomes and plays a key role in TGF- β signal transduction through the recruitment of receptor activated R-Smads for phosphorylation by the type I TGF- β receptor.

► Transforming Growth Factor-Beta

Sarafotoxin

Sarafotoxin is a 21-amino acid peptide which has some structural similarity with endothelins and was isolated from the venom of the burrowing asp. Sarafotoxin is a selective agonist of the endothelin ET_B-receptor.

► Endothelins

SAR-by-NMR

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Synonyms

Lead discovery by NMR; NMR-screening

Definition

NMR can be used as a method for monitoring protein-ligand interactions, either by detecting protein signals or the signals of the ligand. The detection of protein signals requires isotope labeling, usually with ¹⁵N, whereby ligand observed techniques maybe applied without any labeling. Ligand observed techniques are commonly used for screening purposes with the ability to detect binding in the micro- and millimolar range. NMR techniques applied for this purpose are the so called “waterLOGSY” experiment [1], saturation transfer difference spectroscopy [2], and the T1 ρ experiments [2]. The saturation transfer difference technique is often used to determine pharmacophores of the binding ligand. For design purposes, protein observed techniques are preferred since they allow the application of the so called SAR-by-NMR methodology.

SAR-by-NMR is a method for generating systematically lead compounds in the early stages of a drug finding process. NMR is used as a method for detecting protein ligand interactions site-specifically through the application of correlation spectroscopy [3]. The inherent parameter of NMR signals, the chemical shift, is dependent on the chemical environment of the respective nucleus that gives rise to the signal. Binding of a ligand close to this nucleus changes the chemical environment and therefore also the chemical shifts. The monitoring of chemical shift changes through correlation spectroscopy indicates thus the binding site. This information can be used to either i) search for two compounds that bind close to each other in the active center of a protein, and that can be linked chemically to one compound that supposedly binds tighter [3], or ii) to detect the binding of so-called molecular frameworks (small organic compounds) as a basis for the design of focused compound libraries and improvement of the binding affinity of these frameworks by addition of further substitutions to them through a library approach [4]. This procedure is often called “fragment based ligand design.”

Both approaches outlined deliver information that is best used in cycles of medicinal chemistry/computer aided drug design in order to refine the compounds.

In its original form (version i), SAR-by-NMR involved the following steps:

Screening of a compound library to detect compounds that bind to the active site of the protein by correlation spectroscopy.

Screening of derivatives of these compounds, optional a synthesis of a focused library yielding such derivatives to obtain compounds with improved affinity.

Screening for a second compound that binds in the vicinity of the first one using the same procedure.

Determination of a three-dimensional structure of the protein with both ligands.

Design of a linker for both ligands and synthesis of this compound.

Detection of the affinity of the new compound and further improvement by synthesis of focused library on the basis of the design.

Typically, between 1,000 and 10,000 substances are screened to find weakly binding ligands.

The linking of two weaker binding compounds may lead to strong binding one, primarily due to the saving of one entropical factor if both original compounds are chemically connected:

$$\Delta G_{AB} = \Delta G_A + \Delta G_B + \Delta G_{\text{link}}$$

The binding constant increases then:

$$K_d(AB) = K_d(A) * K_d(B) * L,$$

where L is the linking coefficient derived from ΔG_{link} .

In its modified form ii, the first three steps of the original procedure as outlined above are applied to obtain a good starting point for the chemists to synthesize new compounds in a more focused manner.

Description

The NMR screening technology in general is a tool to detect interactions between ligands and proteins in the milli- to picomolar range, using a direct binding assay that detects binding in a site specific manner.

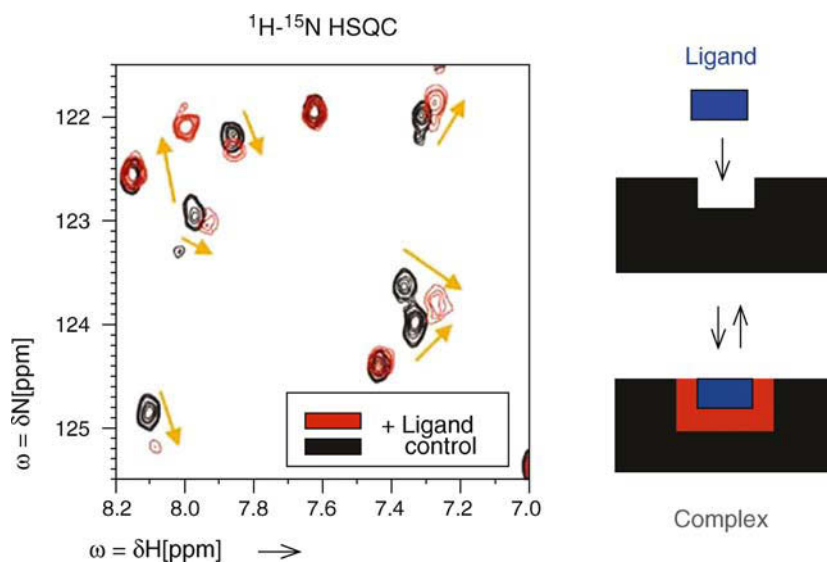
In both variants of the approach, i) and ii), the ligands detected in a NMR screen would be subjected to further modifications, and become larger during the design process. The properties of the compound library need to fulfill therefore a number of NMR-specific requirements. The compounds need to be soluble in water, their molecular properties should not exceed half the number that is defined in the Lipinski rules for molecular

weight, hydrogen bond donors and acceptors. This allows to generate still pharmacologically relevant molecules that satisfy the Lipinski rules at the end of the design process.

Most commonly, protein ligand interactions are monitored by ^1H - ^{15}N correlation spectroscopy (Fig. 1), requiring ^{15}N -labeled protein and approximately 10 min of measurement time for each spectrum. These correlation spectra are particularly well suited for the detection of the binding site, as each amino acid in the protein leads to exactly one cross peak caused by its backbone amid moiety in a usually well-resolved spectrum. Only few amino acids, like arginine, asparagine, glutamine and lysine show additional NH correlation peaks due to the presence of such moieties in the side chain. The correlation signals in ^1H - ^{15}N correlation spectra are the so-called finger print of a protein, since the ^1H and ^{15}N chemical shifts depend on the protein sequence and structure, and occur therefore on individual positions, whereby the secondary structure has a systematical influence on peak position. Due to the occurrence of only a few signals, such spectra are usually also well resolved. Upon binding of a ligand (Fig. 1, right) only those signals in the spectrum of the undistorted protein (black) show chemical shift changes, which are in the direct neighborhood of the ligand in the complex (red). Chemical shift changes are usually quantified as a linear combination of $\delta^1\text{H}$ and $\delta^{15}\text{N}$, e.g.

$$\Delta\delta(^1\text{H}, ^{15}\text{N}) = \Delta\delta(^1\text{H}) + \Delta\delta(^{15}\text{N})/5$$

In principle, any type of NMR spectroscopy may be used to detect the chemical shift changes upon ligand binding. For very small proteins, 1D-NMR might even



SAR-by-NMR. Figure 1 Overlay of regions from ^1H - ^{15}N correlation spectra of a protein without (*black*) and with (*red*) bound ligand (*left*). In case of ligand binding, only signals in the vicinity of the binding sites show chemical shift changes (*red, right*).

be sufficient. Other two-dimensional techniques, such as ^1H - ^{13}C correlation spectroscopy or 2D-TOCSY might be useful in individual cases.

Screening of compound libraries with medium throughput is only possible if the spectra can be recorded in a short period of time, and if one measurement gives simultaneously a number of answers. In practice, several ligands are usually measured in one protein sample, depending on the problem [5]. Mostly 10–20 ligands are combined to multiplexes, which need to be deconvoluted if a positive answer, as shown in Fig. 1 is obtained. The hits obtained maybe analyzed automatically or by hand through manual inspection of the spectra.

Several ligands are often found to interact with the protein, but showing different binding modes. An analysis concerning the binding modes of groups of ligands is possible through a principle component analysis that is used to cluster the results according to concerted chemical shift changes in the respective correlation spectra [6].

The chemical shift changes observed in correlation spectra may be used for measuring binding constants. The position of the protein signal in the absence of a ligand is the concentration of the free protein, whereas an excess of ligand shifts the signal to the position of the one in the complex. A binding curve is the result that maybe evaluated by applying standard methods. The data are conveniently fitted with the following equation:

$$K_D = \frac{([P]_0 - x)([L]_0 - x)}{x}$$

$[P]_0$ is the total molar concentration of the protein and $[L]_0$ is the total molar concentration of ligand. x is the molar concentration of the bound species determined according to the chemical shift change:

$$x = \frac{\delta_{\text{obs}} - \delta_{\text{free}}}{\Delta}$$

δ_{obs} and δ_{free} are the chemical shift values for the target molecule determined at each concentration of ligand and for the protein in the absence of ligand, respectively. Δ is the difference between the chemical shift at saturating amounts of ligand and δ_{free} .

In the case of tighter binding (nanomolar range), the signal of the free protein would not start to change its position upon addition of a ligand, but rather decrease in intensity, and at the position of the chemical shift of the respective resonance in the complex, a signal would appear and its intensity rise with increasing ligand concentration. This may also be plotted as a binding curve.

Examples of the application of SAR-by-NMR include the design of stromelysin and human papillomavirus E2 protein inhibitors [7, 8].

A detailed analysis of designed inhibitors for stromeolysin by thermodynamical methods [7] showed

that the combination of two ligands, a biphenyl derivative and acetohydroxamic acid, yield an increase in the enthalpic contributions to the binding energy, whereas the entropical factor remained constant. In the application of the method to the design of the virus E2 protein the potential of NMR to detect low affinity binding was exploited by starting a ligand refinement from compounds that bound in the millimolar range, and combining features of two compounds to one which finally showed an IC 50 of 10 μM in the applied test.

- Bioinformatics
- Combinatorial Chemistry
- Molecular Modeling

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Sarcolemma

The sarcolemma is the plasma membrane of muscle cells.

- Cardiac Glycosides
- Antiarrhythmic Drugs

Sarcoplasmic Reticulum

Synonyms

SR

Definition

Sarcoplasmic reticulum (SR) is a form of the smooth-faced endoplasmic reticulum (ER) in muscles. It functions as an intracellular Ca^{2+} store for muscle contraction. Ca^{2+} is energetically sequestered into the SR by Ca^{2+} -pump/sarcoplasmic endoplasmic reticulum Ca^{2+} -ATPase (SERCA) and released via Ca^{2+} release channels on stimuli (ryanodine receptor in striated muscles and inositol 1,4,5-trisphosphate receptor in most smooth muscles). Endoplasmic reticulum in non-muscle tissues also functions as an intracellular Ca^{2+} store.

In striated muscles, SR is well developed to surround the myofibrils and is divided into two parts, the terminal cisternae (TC) and longitudinal tubules (LT). TC forms triad (skeletal muscle) or dyad (heart) structure with transverse tubules. The ryanodine receptor is located only in the TC, whereas the Ca^{2+} pump/SERCA is densely packed in both TC and LT.

- Ryanodine Receptor
- Inositol 1,4,5-trisphosphate Receptor
- Endoplasmic Reticulum

SARMs

- Selective Androgen Receptor Modulators

Saxitoxin

Saxitoxin (STX) is a toxin which is found in marine microorganisms. It is most likely synthesized by bacteria which live in symbiosis with dinoflagellates, a component of phytoplankton. Through the marine food chain, it can lead to poisoning of humans. The mechanism of toxicity of saxitoxin is very similar to that of tetrodotoxin. Saxitoxin binds from the outside of the membrane to various forms of voltage-sensitive Na^+ channels and blocks the channel in an activation state-independent manner.

- Voltage-dependent Na^+ Channels

Scaffold

- Adaptor Proteins

Scaffolding Proteins

Scaffolding proteins are proteins, which are able to bind several other proteins in order to organize these interacting proteins into functional complexes. Scaffold proteins often guide the interactions between components in such complexes. This allows, for instance in signaling cascades, a precise relay of the signal with high speed and efficiency. Scaffolding proteins also avoid unwanted cross talk between different functional protein complexes.

In *Drosophila* eyes, the light-activated TRP channel is organized into a supramolecular complex, the “transducisome”, along with other transduction proteins, such as phospholipase C (PLC) and protein kinase C, through association with the scaffolding protein INAD, a multi PDZ domain-containing protein. The mammalian TRPC4 is associated with calmodulin, the inositol 1,4,5-trisphosphate (IP_3)-receptor and, via the scaffolding protein ezrin-binding phosphoprotein 50 or NHERF, with PLC and the actin cytoskeleton.

- AKAPs
- Adapter Proteins
- Phosphodiesterases
- TRP Channels

Scatchard Plot

A Scatchard plot is a plot of B/x against B (where B is the amount of bound ligand and x is the ligand concentration), which is used to estimate the maximal binding, B_{max} as well as the binding affinity (K).

Scavenger Receptors

Cell-membrane proteins that endocytose oxidatively or otherwise modified low-density lipoproteins.

- ▶ Atherosclerosis
- ▶ Lipoprotein Metabolism

Schild Analysis

Schild analysis is a very powerful method to quantify the potency of a competitive antagonist and to test whether the blockade of response by a molecule is consistent with simple competitive antagonism. Devised by Arunlakshana and Schild (1959), it is based on the principle that the antagonist-induced dextral displacement of a dose-response curve is due to its potency (K_{eq} value, affinity) and its concentration in the receptor compartment. Since the antagonism can be observed and the concentration of antagonist is known, the K_{eq} (denoted K_B for antagonist) can be calculated. The relationship between antagonism and concentration must be log-linear with a unit slope to adhere to true competitive kinetics.

- ▶ Drug–Receptor Interaction

Schizophrenia

Schizophrenia is a psychiatric disorder characterized by a number of psychotic manifestations including delusions, hallucinations, disorganized thinking and behavior, amotivation, paucity of thought and speech, flat affect and anhedonia. The aetiology of schizophrenia is thought to involve a combination of genetic and environmental factors. While florid symptoms most commonly present in adolescence or early adulthood, they are usually preceded by premorbid changes in personality and social functioning. Diagnosis is made after exclusion of other causes of psychotic symptoms (e.g., head injury, cerebrovascular accident, substance intoxication) and based on the presence of at least two clinical manifestations present for at least 6 months in the presence of social dysfunction (DSM-IV criteria). Classical antipsychotic drugs (dopamine D2-like receptor blockers) are effective in reducing the positive symptoms of schizophrenia (delusions, hallucinations) but are less useful in treating the negative symptoms of the disorder (loss of affect, withdrawal).

- ▶ Antipsychotic Drugs
- ▶ Dopamine System

Second Messenger

A second messenger is an intracellular metabolite or ion whose concentration is altered when a receptor is activated by an agonist, considered to be the “first messenger.”

Secretase

The enzymes that are involved in the processing of APP into amyloid peptides are known as secretases. Beta-secretase is an enzyme that catalyzes the initial proteolytic event leading to the production of Abeta amyloid peptides. If APP is cleaved by beta-secretase it can then be further cleaved by gamma-secretase. Abeta peptides are either secreted or intracellularly released. They have varying lengths and represent intermediate degradation products of its precursor (i.e., β -secretase cleaved APP). Especially the 42 amino acid peptide ($A\beta_{42}$) aggregates to form “insoluble” amyloid plaques.

- ▶ Alzheimer’s Disease

Secretory Pathway

Transport of proteins and lipids occurs between the organelles of the secretory pathway, i.e. endoplasmic reticulum (ER), Golgi, endosomes, lysosomes and the plasma membrane.

- ▶ Intracellular Transport

Sedatives

Sedatives decrease activity, moderate excitement and calm the recipient. In contrast to hypnotic drugs, they do not produce drowsiness and do not facilitate the onset

and maintenance of sleep. The most important group of sedatives are the benzodiazepines given at low or moderate doses.

► [Benzodiazepines](#)

Seizure

A seizure is an abnormal behavioral (often motoric) activity caused by abnormal electrical activity of the brain. Seizures can be the symptom of a chronic neurological malfunction, i.e. epilepsy, or can appear as single events, e.g. during fever in infants.

► [Antiepileptic Drugs](#)

Selectins

The three selectins are related both structurally and functionally. They are transmembrane proteins, with an N-terminal C-type lectin domain, followed by an EGF repeat and a variable number of complement control protein (CCP) domains. Selectins bind carbohydrates, which are present in various glycoproteins.

- [Inflammation](#)
- [Table Appendix: Adhesion Molecules](#)

Selective Androgen Receptor Modulator

Synonyms
SARM

Definition

SARMs are AR ligands that display tissue-specific androgenic/antiandrogenic activities.

► [Selective Sex Steroid Receptor Modulators](#)

Selective Estrogen Receptor Downregulator

Selective estrogen receptor downregulators (SERDs) is a class of medication that induces an estrogen receptor downregulation.

- [Selective Sex Steroid Receptor Modulators](#)
- [Targeted Cancer Therapy](#)

Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs) are synthetic compounds with partially agonistic and partially antagonistic estrogenic properties. In bone, SERMs inhibit bone resorption via the mechanisms known for estrogens. Major SERMs are tamoxifen, a triphenylethylene compound, and raloxifene. In postmenopausal women, the latter has been shown to prevent bone loss and to reduce fracture risk by 40%.

- [Selective Sex-Steroid Receptor Modulators](#)
- [Targeted Cancer Therapy](#)

Selective Noradrenaline Reuptake Inhibitors

Selective noradrenaline reuptake inhibitors (SNRIs) are a group of drugs, which act as antidepressants by the selective inhibition of the reuptake of noradrenaline from the synaptic cleft via the selective blockade of the noradrenaline-specific neurontransmitter transporter (e.g. reboxetine).

- [Antidepressants](#)
- [Neurotransmitter Transporters](#)

Selective Progesterone Receptors Modulators

Selective progesterone receptor modulators (SPRMs, mesoprogestins) are progesterone receptor (PR) ligands

that exhibit agonistic and antagonistic activities. Examples are J876 and J1042.

► Selective Sex Steroid Receptor Modulators

Selective Serotonin Reuptake Inhibitors

Selective serotonin reuptake inhibitor (SSRI) is an abbreviation for the class of antidepressants known as the Selective Serotonin Reuptake Inhibitors. Examples of SSRIs include fluoxetine, paroxetine, citalopram, and sertraline. These drugs selectively inhibit the serotonin transporter thus prolonging the synaptic lifespan of the neurotransmitter serotonin.

- Antidepressants
- Neurotransmitter Transporters
- Brain Derived Neurotrophic Factor
- Neurotrophic Factors
- Corticotropin Releasing Hormone
- CRH
- Serotonergic System

Selective Sex Steroid Receptor Modulators

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Synonyms

Mixed agonists/antagonists; Tissue-specific agonists/antagonists

Definition

Compounds that mimic the effects of sex steroids in some tissues, while at the same time can oppose endogenous hormone action in other tissues.

Background

The sex steroids comprise a class of hormones, including ►estrogens, ►progestins, and ►androgens, which play important roles in the development and maintenance of

the male and female reproductive systems. In addition, sex steroids have functions in tissues other than those related to reproduction. For example, estrogen is involved in the development and maintenance of skeletal integrity and is an important regulator of triglyceride and cholesterol homeostasis. Not all of the biological functions of the sex steroids are beneficial, however, as chronic stimulation by estrogens, progestins, and androgens has been implicated in the genesis and progression of cancers in a number of tissues. The necessity for compounds that retain the beneficial effects of sex steroids in some tissues but oppose the action of endogenous hormones in others has resulted in the generation of a novel class of pharmaceuticals termed ►selective steroid receptor modulators (SSRMs). This chapter will focus primarily on known ►selective estrogen receptor modulators (SERMs), which represent a class of pharmaceuticals used clinically for cancer and osteoporosis, and for sustaining beneficial effects of estrogen in postmenopausal women [1]. The general mechanisms of SERMs are similar to that of ►selective progesterone receptor modulators (SPRMs) and ►selective androgen receptor modulators (SARMs), which are currently in development for the treatment of progesterone- and androgen-associated pathologies, respectively.

Mechanism of Action

Targeting Estrogen Action in a Tissue-Specific Manner: The SERM Concept

The ovarian hormone estrogen is a key regulator of the processes involved in the growth, differentiation and function of a wide variety of tissues of diverse functions. The importance of estrogen in sustaining overall health is evidenced by the adverse effects of hormone deficiency in postmenopausal women who experience increased hot flashes, depression, cardiovascular disease, and losses in bone mineral density that often lead to osteoporosis. These observations have led to the development of ►estrogen replacement therapies (ERT), where premenopausal estrogen levels are restored, and the symptoms of menopause are alleviated. The most well studied responses are in bone, where ERT can decrease the incidence of hip fractures by 50%, and in the central nervous system, where up to 90% inhibition of hot flashes is observed. ERT has also been shown to be associated with improvement in cognitive function in postmenopausal women and may delay the onset of Alzheimer's disease [1].

Although the positive effects of ERT have been well established, it has been shown that the cell proliferative actions of estrogen can increase the incidence of breast cancer in some patients. In addition, duration of exposure to physiological levels of unopposed estrogens is an established risk factor for breast, uterine, and ovarian cancer. In an effort to attain pharmaceutical agents that oppose the carcinogenic

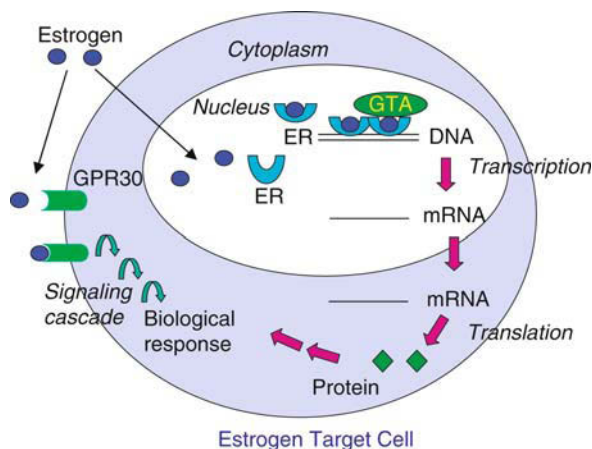
actions of estrogens, ►**antiestrogens** have been developed, and are being used clinically for the treatment of ►**estrogen receptor (ER)**-positive breast cancers. The necessity for compounds that retain the beneficial effects of estrogen in some tissues has resulted in the generation of a novel class of antiestrogens, (SERMs), which display tissue-specific ►**agonist** and ►**antagonist** activities [1].

Molecular Mechanisms of SERM Action as a Means of Understanding their Tissue-Selective Activities

The molecular pharmacology of estrogens and antiestrogens is complex. It is clear, however, that all of these compounds mediate their biological activities through two intracellular receptors, the ERs (ER α and ER β) which function as ligand-inducible transcription factors in target cell nuclei [1]. Hormone binding to the receptors transduces the endocrine signal into genomic responses, resulting in the upregulation or downregulation of specific genes at the messenger RNA (mRNA) level. The mRNAs are then translated into proteins that function within the cells of hormone-responsive tissues to regulate proliferation, differentiation, and homeostasis (Fig. 1).

In addition to the well-established genomic signaling pathway through ER α and ER β , estrogens have recently been shown to display rapid responses that do not require gene transcription. This 'nongenomic' signaling is associated with estrogen activation of other receptor classes such as membrane associated receptor tyrosine kinases and G protein coupled receptors (GPCR), such as the recently described ►**GPCR**, ►**GPR30** [2]. Specific biological functions of this receptor have yet to be defined, however, the ability of GPR30 to bind both classical ER agonists and antagonists suggests that GPR30 may play a role in antiestrogen pharmacology [2].

Ligand binding is an event that initiates ER signaling. According to classical receptor theory, agonists (such as endogenous estrogens) act as molecular switches, converting ER from an inactive to an active form. Antiestrogens, synthetic compounds developed to oppose the action of natural hormone, were thought to competitively inhibit agonist binding and in doing so were able to lock the receptor in a latent state. Thus, it was considered that when corrected for affinity all agonists were functionally indistinguishable, and likewise, antagonists were all the same. However, the existence of SERMs indicated that this model is oversimplified, as it does not account for the biology of known antiestrogens. Some of the first evidence that antiestrogens do more than freeze ER in a latent state and thus play a more active role in ER action came from clinical studies of patients that were administered ►**tamoxifen** as adjuvant therapy for estrogen-dependent breast tumors. Strikingly, while tamoxifen blocked the



Selective Sex Steroid Receptor Modulators.

Figure 1 The biological effects of estrogens are mediated through estrogen receptors (ERs). Hormone diffuses into target cells where it binds to nuclear ERs. This event induces a conformational change in ER that enables the receptor to bind to the regulatory region of estrogen-responsive genes. The DNA-bound ER interacts with components of the general transcriptional apparatus (GTA) to induce gene transcription. The resultant mRNAs are transported into the cell cytoplasm where they are translated into proteins. These proteins function with the cell to mediate biological responses. In addition to classical nuclear ER-mediated pathways, nongenomic, rapid actions of estrogens have recently been identified. Estrogen binds cell surface receptors such as membrane-associated ERs or GPR30, a G-protein activated receptor. Hormone binding to either membrane ERs or GPR30 can activate downstream kinase signaling pathways. The biological consequences of nongenomic estrogen signaling are currently under investigation.

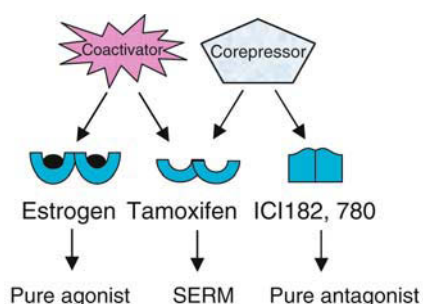
actions of estrogen in breast cancer cells, it was shown to function as an agonist in bone and the uterus, mimicking the actions of estrogen. The observation that tamoxifen displays tissue-specific agonist/antagonist activities was inconsistent with the classical definition of antagonist action, and furthermore, suggested that this compound may alter ER in such a way that the liganded receptor would be recognized differently in distinct cell types [1].

The concept that different ligands play an active role in ER function is apparent at the biochemical level. In addition to competitive inhibition of estrogen binding, antiestrogens induce unique conformations/structures of both ER α and ER β . This provides a structural basis for the unique biological activities displayed by the different compounds [1].

The understanding of diversity in ligand-mediated ER activity was advanced by the discovery of transcriptional cofactors. These proteins, termed ►**coactivators** and ►**corepressors**, bind the liganded ER and enhance

or decrease ER-mediated transcription of target genes, respectively. The ability of different ER agonists and antagonists to induce different conformations of the receptors influences the binding of coactivators and corepressors to the receptors (Fig. 2). Different ligands induce unique structural changes in ER that result in differential recruitment of coactivators and corepressors, leading to diversity in biological response. This provides a mechanism by which different ligands acting through the same receptor can mediate unique biological effects [1]. For example, estrogen induces coactivator recruitment to ER, whereas when bound to the pure antiestrogen ICI 182,780, (A pure ER antagonist that opposes estrogen action in all tissue contexts.) the conformation of ER is compatible with corepressor (but not coactivator) binding. Correspondingly, estrogen is a full agonist of ER, while ICI functions as a pure antagonist on the receptor. In contrast, when bound to the SERM tamoxifen, which displays both agonist and antagonist activities, ER is capable of interacting with either coactivators or corepressors. It is likely that diversity in the availability of coactivators or corepressors in different target cells may be the mechanism underlying the tissue-specific agonist/antagonist activities of tamoxifen and other SERMs. Thus, it will be important to identify the cofactor proteins present in different ER target cells in order to allow mechanistic screening for new tissue-targeted SERMs.

Interestingly, the antagonist activity of ICI and other antiestrogens has also been recently attributed to the ability of these compounds to induce degradation of the ER protein in estrogen target tissues. It is thought that



Selective Sex Steroid Receptor Modulators.

Figure 2 Cofactor binding is regulated by the structure of the ligand–ER complex. Different ligands induce distinct conformations of ER, leading to differential cofactor recruitment. Pure agonists like estrogen, drive the receptor into a structure compatible with coactivator binding, whereas pure antagonists (ICI182,780) induce a conformation recognized by corepressors. SERMs like tamoxifen, which display both agonist and antagonist activities on ER, permit the receptor to interact with either coactivators or corepressors.

coactivator and corepressor recruitment to the ER is involved and may facilitate receptor degradation, however, further investigation is needed to define the specific cofactor complexes involved [3].

Clinical Use

Mechanistic Classes of Antiestrogens and SERMs

ER antagonists comprise two broad categories: pure antiestrogens and SERMs. A summary of the different biological functions associated with some of the known antiestrogens is displayed in Table 1.

Pure Antiestrogens: Pure antiestrogens, represented by ICI 182,780, oppose estrogen and ER activity in all tissues [1]. In theory, these properties make Type I antiestrogens useful in cancer therapy, since they block the carcinogenic effects of estrogen in the breast and reproductive system. However, since ICI 182,780 lacks the beneficial agonist effects of estrogen in bone, the cardiovascular, and the central nervous systems, the clinical use of this compound is limited to patients with advanced breast cancer who have become resistant to tamoxifen (see below).

SERMs:

1. **First Generation:** The triphenylethylene tamoxifen (Nolvadex) is the most well-characterized SERM, used clinically since 1971. The notable antagonist effects of tamoxifen in the breast make it a first line therapy for the treatment of pre- and postmenopausal women with estrogen-responsive advanced (Stage IV) breast cancer. In addition to the use in the prevention and treatment of cancer, tamoxifen has very beneficial effects in the maintenance of bone mineral density and decreases serum triglyceride (LDL) levels in postmenopausal women. Unfortunately, the estrogenic activity of tamoxifen provides for undesirable uterotrophic effects, leading to an increased risk for endometrial cancer in women undergoing prolonged tamoxifen therapy. Similar to that seen in women on ERT, tamoxifen also induces ovarian cysts due to its agonist activity in the ovary.

Selective Sex Steroid Receptor Modulators.

Table 1 Biological activities of ER ligands in selected target tissues

	Bone	Breast	Cardiovascu- lature	Uterus
Estrogen	+++	+++	+++	+++
IC1182,780	–	–	–	–
Tamoxifen	+	–	+	+
Raloxifene	+	–	+	–
Lasofixifene	++	–	++	–

+ denotes agonist activity; – denotes antagonist activity

An additional concern has been the observed development of “tamoxifen resistance” in a significant proportion of women after 5 years of antiestrogen therapy. This term refers to the phenomenon by which certain breast cancers alter their biology and recognize the compound as an agonist for growth. Other tamoxifen-derived compounds such as toremifene, droloxifene and idoxifene are currently being evaluated in clinical studies for their potential long-term beneficial effects in antagonizing ER-positive breast tumor growth [1].

2. **Second Generation:** Second generation SERMs were developed with the objective of obtaining ER-targeted pharmaceuticals that lacked the uterotrophic and carcinogenic effects of tamoxifen. The most well characterized second generation SERM is **raloxifene** (EVISTA). This compound functions as an estrogen in bone and the cardiovascular system, but acts as a pure antagonist in the breast and uterus. Type II antiestrogens are currently in clinical use; raloxifene was shown to prevent bone loss in preclinical trials of postmenopausal osteoporosis. Consequently, raloxifene received FDA approval for the prevention and treatment of osteoporosis in 1999 and is now widely used in the clinic [1, 3]. The **STAR trial** (Study of tamoxifen and raloxifene), which was completed in 2006, demonstrated additional utility of raloxifene in the prevention and treatment of breast cancer. In fact, the absence of associated uterotrophic effects with raloxifene suggests that it may be a safer agent than tamoxifen for use as a chemopreventative in high-risk postmenopausal women [3]; therefore, raloxifene has very recently become a new option for breast cancer prevention now available for physicians and their patients.
3. **Third Generation:** Third generation SERMs are ER-targeted pharmaceuticals that possess the favorable qualities of raloxifene, but are more effective in their estrogenic effects of enhancing bone mineral density and in decreasing serum LDL and cholesterol levels. Many of these compounds, such as **bazedoxifene** and **lasofoxifene** are presently being evaluated in clinical trials for their efficacy in the prevention of osteoporosis [3].

Quest for the “Perfect” SERM

Recently, leaders in the pharmaceutical industry have developed a list of desired properties for a fourth generation of SERMs (Table 2). In general, future SERMs must oppose endogenous hormone action in the breast and reproductive system while displaying full estrogenic effects in the cardiovascular, bone and central nervous systems. Additional criteria are that fourth generation compounds possess superior bioavailability compared with existing SERMs and have

Selective Sex Steroid Receptor Modulators.

Table 2 Criteria for fourth generation SERMs

1	Antagonize estrogen action in the breast and ovary
2	Display no uterotrophic activity
3	Bone protective to the full extent of estrogen
4	Possess better cardiovascular and central nervous system profiles than current SERMs
5	Possess superior bioavailability than current SERMs
6	Contain potential benefits for men in protection against age-related bone loss and increases in cholesterol levels, without displaying estrogenic proliferative effects in the prostate

additional indications in men for bone protection and cardioprotection.

In addition to those described above, some of the newest compounds emerging in SERM development are ER β -selective ligands and pathway-selective modulators that target the interaction of the ERs with the transcription factor NF κ B. While such compounds are in the early stages of clinical evaluation, thus far they demonstrate great potential for use in the treatment of inflammatory disorders such as arthritis, inflammatory bowel disease, and like other SERMs, cancer [4].

Selective Progesterone Receptor Modulators (SPRMs)

Progestins (progesterone and related compounds) are ovarian hormones that mediate their biological effects through an intracellular **progesterone receptor (PR)**, a hormone-inducible transcription factor. Progesterone is involved in the development of the female reproductive system, pregnancy and fertility, and in regulation of gonadotropin hormone secretion. Progestins are used clinically in oral contraceptives, to induce abortion and missed menses, and in treatment of endometriosis and fibroids [1]. Since progestins function as antiestrogens in the uterus, yet display proliferative effects in the breast, pharmaceutical companies are currently engaged in efforts to develop compounds that display tissue-targeted progestin/antiprogestin activities. This should provide for an additional class of anticancer agents that retain the beneficial effects of progesterone in selected tissues.

The first SPRM to reach the advanced stage of clinical development for treatment of **endometriosis** and uterine fibroids, **asoprisnil**, is expected to receive FDA approval this year. The therapeutic effect of asoprisnil stems from its PR antagonist/antiproliferative activity in the endometrium and breast. Unlike classical PR antagonists however, this compound does not induce labor in animal models of pregnancy and parturition. Recent structural studies

demonstrated that asoprisnil-bound PR resides in a different conformation than PR complexed with progesterone. These structural differences might explain the agonist/antagonist behavior of this compound, as in contrast to progesterone or PR antagonists, asoprisnil facilitates recruitment of both coactivators and corepressors to PR [5].

Selective Androgen Receptor Modulators (SARMs)

Androgens (testosterone and related compounds) comprise a class of male steroid hormones secreted by the testes. Androgens are essential for spermatogenesis, formation of reproductive organs and development of secondary sexual characteristics. In addition, androgens are involved in bone and muscle growth, regulation of hypothalamic–pituitary hormone secretion, reproductive behavior, and neural regeneration [1]. Unfortunately, prolonged exposure to unopposed androgens is an established risk factor for prostate cancer, a serious health concern among middle-aged and elderly men. The proliferative effects of androgens in the prostate are mediated through the **▶androgen receptor (AR)**, which like ER and PR, functions as a hormone-inducible transcription factor in target cells. AR antagonists (antiandrogens) are currently in development and clinical use for prostate cancer, as they oppose the proliferative actions of androgens in the prostate [1].

Tissue-targeted antiandrogens termed SARMs are currently in the early stages of development, and preclinical data is very promising. The high anabolic activity in selective tissues such as muscle and bone indicates that SARMs could be used in the treatment or prevention of many diseases such as muscle wasting, osteoporosis, frailty, or other conditions that are a consequence of aging or androgen deficiency. Two other indications for SARMs are currently under investigation: (i) partial anti-androgenic activity of some compounds in the prostate suggests their utility in benign prostate hyperplasia and (ii) the identification of agents that selectively inhibit spermatogenesis may provide a novel hormonal means of male contraception.

Summary

The sex steroids comprise a family of hormones, which share a similar mode of action through binding nuclear receptors and regulating the expression of genes involved in cell proliferation, differentiation and function. Sex steroids have a wide range of biological roles in reproduction and in other organ systems. While many hormone functions are critical for homeostasis, chronic stimulation of sex steroid-mediated pathways has also been implicated in cancer and other pathologies. The necessity for compounds that retain the beneficial effects of sex steroids in some tissues

but oppose the action of endogenous hormones in others has resulted in the generation of SSRMs, which display tissue-specific agonist and antagonist activities. Further investigation into the mechanisms by which different cells recognize liganded–receptor complexes in a unique manner will provide for mechanistic screening for new pharmaceuticals that display more specific and effective tissue-targeted activities.

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Self Tolerance

During differentiation of T- or B-lymphocytes[®] antigen receptors are generated which react to selfor[®] autoantigens. These are generally eliminated by the mechanisms of central tolerance or kept silent by the mechanisms of peripheral tolerance ([®] autoimmune disease).

▶Autoimmune Disease

Sema Domain

The Sema domain consisting of about 500 amino acids is characterized by highly conserved cysteine residues that form intramolecular disulfide bonds. Crystal structures have revealed that the Sema domain folds in the manner of the β propeller topology which is also found in integrins or the low-density lipoprotein (LDL) receptors. Sema domains are found in semaphorins, plexins and in the receptor tyrosine kinases Met and Ron.

▶Plexins

Semaphorins

Semaphorins are secreted, membrane-associated or transmembrane proteins defined by the presence of a semaphorin protein domain (Sema domain). In the mammalian system, more than 20 semaphorins have been identified which play important roles in a variety of tissues. The best characterized receptors for mediating semaphorin effects are members of the neuropilin and plexin families of transmembrane proteins. Semaphorin functions are best described in the regulation of neural development, angiogenesis, immunoregulation and cancer.

► Plexins

Senescence

Senescence is defined as cellular ageing resulting in an irreversible cell cycle arrest. Primary cells divide about 50 times and then are arrested due to senescence. Senescence is associated with shortening of telomeres.

► Cell Cycle Control ► Telomerase

Sensitization

Sensitization has been implicated in the development of compulsive drug use and involves a dramatic augmentation of behavioral and neurochemical responses associated predominantly with mesolimbic dopamine transmission that often develops with intermittent exposure to drugs of abuse. Whether sensitization of mesolimbic dopamine neurons is linked to enhanced rewarding efficacy remains unclear. Indeed, current conceptualizations of the significance of sensitization in compulsive drug-seeking behavior hold that, rather than enhancing “reward,” repeated drug use leads to a progressive and persistent hypersensitivity of neural systems that mediate “incentive salience” resulting in excessive craving.

► Drug Addiction/Dependence

Sensory Nerves

Sensory nerves are peripheral nerves involved in the detection of exogenous and endogenous stimuli for transmission into the CNS. They can be subdivided into RARs and C-fibres according to their channel characteristics.

► Antitussive Drugs ► Nociception

Sentrin

► SUMOylation

Septicemia

Septicemia is generally a serious illness caused by the presence of bacteria and/or bacterial toxins in the blood (blood poisoning).

► Anticoagulants

Sequence Contig

A contig consists of a set of gel readings from a sequencing project that are related to one another by overlap of their sequences. The gel readings of a contig can be combined to form a contiguous consensus sequence whose length is called the length of the contig.

► Bioinformatics

Sequence Profile

A sequence profile represents certain features in a set of aligned sequences. In particular, it gives

position-dependent weights for all 20 amino acids and as for insertion and deletion events at any sequence position.

► [Bioinformatics](#)

Sequence-specific Transcription Factors

Proteins that bind DNA at specific DNA sequences often distal from transcriptional start sites of genes. Their binding and activity is usually cell type or stimulus triggered, which subsequently decondensate the chromatin and finally lead to the recruitment of general transcription factors and the RNA polymerase.

► [Transcriptional Regulation](#)

Sequential Preparations

Sequential preparations contain only estrogens in the first and a fixed estrogen/progestin combination in the second phase of the application period.

► [Contraceptives](#)

SERCA

Sarcoplasmic calcium ATPase; this enzyme utilizes the energy gained from hydrolysis of ATP to pump calcium from the cytosol into the stores of the sarcoplasmic reticulum. Its activity is negatively regulated by the closely associated protein phospholamban, and this inhibition is relieved upon phosphorylation of phospholamban by protein kinase A (PKA).

Serine-hydroxymethyltransferase

The amino acid glycine, a neurotransmitter at inhibitory synapses throughout the central nervous system (CNS),

is preferentially synthesized by the tetrahydrofolate and pyridoxal phosphate-dependent enzyme serine-hydroxymethyltransferase. In the CNS, glycine synthesis involves the mitochondrial isozyme. While serine-hydroxymethyltransferase may also contribute to glycine catabolism, the preferred degradation is via the glycine cleavage system, resulting in the formation of CO₂ and ammonia.

► [Glycine Receptors](#)

Serine Proteinases

Serine proteinases are proteinases that utilise the terminal hydroxyl group of the side chain of serine to affect peptide bond hydrolysis.

► [Non-viral Peptidases](#)

► [Proteinase-activated Receptors](#)

Serine/Threonine Kinase

► [Table appendix: Protein Kinases](#)

SERM

Selective Estrogen Receptor Modulators.

► [Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor](#)

Serotonin

Serotonin or 5-hydroxytryptamine is an important biogenic amine, which is synthesized via 5-hydroxytryptophan from the amino acid tryptophan. The highest concentration of serotonin occurs in the wall of the intestine. About 90% of the total amount is present in enterochromaffin cells, which are derived from the neural crest, similarly to those of the adrenal medulla.

Enterochromaffin cells are interspersed with mucosal cells mainly in the stomach and small intestine. In the blood, serotonin is present at high concentrations in platelets, which take up serotonin from the plasma by an active transport process. Serotonin is released on platelet activation. In the central nervous system, serotonin serves as a transmitter. The main serotonin-containing neurons are those clustered in form of the Raphe nuclei. Serotonin exerts its biological effects through the activation of specific receptors. Most of them are G-protein coupled receptors (GPCRs) and belong to the 5-HT₁-, 5-HT₂-, 5-HT₄-, 5-HT₅-, 5-HT₆-, 5-HT₇-receptor subfamilies. The 5-HT₃-receptor is a ligand-operated ion channel.

- Serotonergic System
- Psychotomimetic Drugs

Serotonergic System

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Synonyms

Serotonin = 5-Hydroxytryptamine = 5-HT (=enteramine)

Definition

The serotonergic system is one of the oldest neurotransmitter/hormone systems in evolution, which may explain why ►5-HT interacts with such a diversity of receptors of the G protein-coupled family and the ligand-gated family, similarly to acetylcholine, GABA or glutamate. 5-HT was discovered in the gut in the 1930s and called enteramine, then rediscovered in the 1940s in the blood and called serotonin, since it constricted smooth muscle in vessels and gut. 5-HT is synthesized from L-tryptophan, there are two tryptophan hydroxylases forming 5-hydroxytryptophan (5-HTP), which by the L-amino acid decarboxylase leads to 5-HT; serotonin can be conjugated with glucuronide or sulfate or in the nervous system, metabolized via monoamine oxydase to 5-hydroxyindolacetaldehyde and finally to 5-hydroxyindolacetic acid (via aldehydedehydrogenase). It can also lead to 5-hydroxytryptophol by an aldehydereductase in some peripheral nerves. Thus, 5-HT acts both as a neurotransmitter with all the features, such as intracellular storage, activity-dependent release, the existence of both pre- and post-synaptic receptors, an active uptake

system, via the serotonin transporter and metabolizing/inactivating enzymes and a hormone, released into the blood or gut to work more distantly.

Basic Characteristics

Physiology

The main source of 5-HT (95%) is in the enterochromaffin cells of the gut, where it is synthesized from tryptophan. It can be released into the gut lumen, e.g. as a reaction to pressure and act on receptors located on the smooth muscle, or into the portal blood circulation, by a variety of nervous or alimentary stimuli. 5-HT is also found in enteric neurons. In the blood, the vast majority of 5-HT is found in storage granules in the platelets, which are endowed with a very active uptake system (they probably do not synthesize 5-HT). Large amounts of 5-HT are released during platelet aggregation, and it can act locally on endothelial cells and vascular smooth muscles. 5-HT is also found in mast cells. In the central and peripheral nervous system, 5-HT acts as a neurotransmitter on a large variety of receptors, which may be located pre- or post-synaptically. 5-HT is also found in the pineal gland, where it is believed to serve essentially as a precursor for the synthesis of melatonin by 5-HT-*N*-acetyltransferase and hydroxyindole-*O*-methyltransferase, under the control of the clock genes in the suprachiasmatic nucleus, which during the circadian rhythm modulates enzyme activity levels up to 50 fold.

Multiple 5-HT Receptor Subtypes

There are at least 15 different 5-HT receptors, and the system is probably more complex (see [Tables 1–3](#) and [Fig. 1](#)). With the exception of 5-HT₃ receptors, (ligand-gated ion channels), 5-HT receptors belong to the ►G protein-coupled receptor (GPCR) superfamily and represents one of the most complex families of neurotransmitter receptors. Multiple splice variants (5-HT_{2C}, 5-HT₄, 5-HT₇) or RNA edited isoforms (5-HT_{2C}) have been described; there is also evidence that homo- and hetero-dimerization (5-HT_{1B/1D}) can occur. Furthermore, peptide or lipid modulators of 5-HT receptors have been described such as 5-HT moduline (Leu-Ser-Ala-Leu (LSAL), a putative product of a chromogranin), which has selectivity for the 5-HT_{1B} and 5-HT_{1D} receptors, or oleamide, which acts on several receptors (e.g. 5-HT_{2A/2C} and 5-HT₇). As molecular biology started to play a prominent role in the discovery of additional receptors, the Serotonin Club Receptor Nomenclature Committee proposed a classification system based on operational, structural, and transductional information [1]. The current classification [4] was progressively adapted to incorporate new information, obtained from both recombinant and native receptors, and favors an alignment of nomenclature with the human genome to avoid species differences.

Serotonergic System. Table 1 5-HT₁ receptors

Nomenclature	5-HT _{1A}	5-HT _{1B}	5-HT _{1D}	5-HT _{1E}	5-HT _{1F}
Selective agonist	8-OH-DPAT	L 694247	PNU 109291	None	LY 334370, LY344864
Rank order	Di-nPr-5-CT ≥ 5-HT > 8-OH-DPAT > 5-HT > buspirone > sumatriptan	<i>Human</i> : 5-CT > 5-HT > sumatriptan > RU-24969 > CP-93,129 > 8-OH-DPAT <i>Rodent</i> : RU-24969 > 5-CT = CP-93,129 > sumatriptan > 8-OH-DPAT		5-HT > RU-24969 > 5-CT > sumatriptan	
Antagonists (pK _B)	(±)WAY 100635 (8.7)	GR 55562 (7.4) SB 224289 (8.5) SB 236057 (8.9)	BRL 15572 (7.9)	-	-
Radioligands	[³ H]WAY100635	[¹²⁵ I]GTI	[¹²⁵ I]GTI	[³ H]5-HT	[¹²⁵ I]LSD [³ H]LY 334370
Effector	G _i /G _o Preferentially inhibits cAMP formation Increases inwardly rectifying K ⁺ current, PLC activation with increased [Ca ²⁺] _i and elevated [Ca ²⁺] _i observed in recombinant systems	G _i /G _o Preferentially inhibits cAMP formation; PLC activation with increased IPs and elevated [Ca ²⁺] _i to observed in recombinant systems.	G _i /G _o Preferentially inhibits cAMP formation; PLC activation with increased IPs and elevated [Ca ²⁺] _i observed in recombinant systems.	G _i /G _o Preferentially inhibits cAMP formation (in recombinant system).	G _i /G _o Preferentially inhibits cAMP formation (in recombinant system).
Localization	CNS: Hippocampus (CA1, CA3, DG), septum, amygdala, raphe nuclei <i>Peripheral</i> : Cholinergic heteroreceptor - myenteric plexus	CNS: Striatum, hippocampus (CA1), substantia nigra, globus pallidus, superior colliculi, spinal cord, raphe nuclei <i>Peripheral</i> : Vascular smooth muscle, autonomic terminals	CNS: like 5-HT _{1B} but at lower densities. <i>Peripheral</i> : autonomic and trigeminal nerve terminals	CNS: Caudate putamen, parietal cortex, fronto-parietal motor cortex, olfactory tubercle, amygdala <i>Peripheral</i> : None identified	CNS: Cortex, Thalamus, olfactory bulb (rat), claustrum (g-pig), hippocampus (CA3), spinal cord. <i>Peripheral</i> : Uterus, mesentery

Serotonergic System. Table 2 5-HT_{2, 3, 4} receptors

Nomenclature	5-HT _{2A}	5-HT _{2B}	5-HT _{2C} ^F	5-HT ₃	5-HT ₄
Selective Agonist	DOI	BW 723C86	Ro 600175	SR 57227, mCPB	BIMU 8, SC53116
Rank order		5-HT = α -me-5-HT > BW723C86 > 5-CT = 2-me-5-HT >> sumatriptan	5-HT = α -me-5-HT > DOI > RU-24969 = quipazine > 5-CT > sumatriptan	m-CPB > 5-HT > 2-me-5-HT >> 5-MeOT	ML10302, RS 67506 SC53116 $\geq$ BIMU 8 > 5-MeOT > α -me-5-HT > cisapride > (S)-zacopride > 5-CT
Selective antagonists (pK _B)	Ketanserin (8.5–9.5)	SB 200646 (7.5) ^{† †}	Mesulergine (9.1)	Granisetron (10)	GR 113808 (9–9.5)
	MDL 100907 (9.4)	SB 204741 (7.8)	SB 242084 (9.0)	Ondansetron (8-10)	SB 204070 (10.8)
			RS 102221 (8.4)	Tropisetron (10-11)	RS 100235 (11.2)
Radioligands	[¹²⁵ I]DOI	[³ H]5-HT	[¹²⁵ I]LSD	[³ H](S)-zacopride	[¹²⁵ I]SB 207710
	[³ H]Ketanserin		[³ H]Mesulergine	[³ H]tropisetron	[³ H]GR 113808
	[³ H]MDL 100907			[³ H]granisetron	[³ H]RS 57639
				[³ H]GR 65630	
				[³ H]LY 278584	
Effector	G _{q/11} Preferentially increases Pi hydrolysis and elevates [Ca ²⁺] _i	G _{q/11} Preferentially increases Pi hydrolysis and elevates [Ca ²⁺] _i (in recombinant systems)	G _{q/11} Preferentially increases Pi hydrolysis and elevates [Ca ²⁺] _i	Intrinsic ligand-gated ion channel Promotes increased [Na ⁺] [Ca ²⁺] _i	G _s Preferentially increases cAMP formation.
Localization	<i>CNS</i> : Cortex, hippocampus, striatum, olfactory bulb, spinal cord	<i>CNS</i> : not present in adult.	<i>CNS</i> : Choroid plexus, medulla, pons, striatum, hippocampus (CA1, CA3), hypothalamus, spinal cord	<i>CNS</i> : Striatum, hippocampus (CA1), substantia nigra, globus pallidus.	<i>CNS</i> : Striatum, brainstem, thalamus, hippocampus, olfactory bulb, substantia nigra
	<i>Peripheral</i> : GI, vascular and bronchial smooth muscle, vascular endothelium, platelets	<i>Peripheral</i> : Smooth muscle of ileum, stomach fundus (rat), uterus, vasculature, endothelium	<i>Peripheral</i> : None identified	<i>Peripheral</i> : Post-ganglionic sympathetic neurons, sensory neurons	<i>Peripheral</i> : Cardiac muscle, post-ganglionic parasympathetic neurons (myenteric plexus), esophageal and vascular smooth muscle

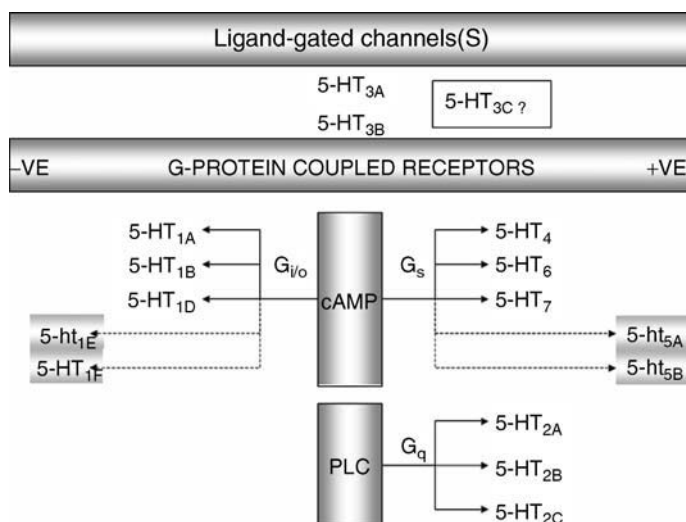
Currently, seven families of 5-HT receptors have been recognized, 5-HT₁ to 5-HT₇.

The 5-HT₁ receptor class comprises five receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}) which, in humans, share 40–63% overall sequence identity and couple somewhat preferentially to G_{i/o} to inhibit cAMP formation (see [Tables 1–3](#)). The 5-HT_{1E} receptors are given a lower case appellation to denote that

endogenous receptors with a physiological role have not yet been found, and may not even exist in rodents (rat, mouse). In contrast, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1F} receptors have been demonstrated functionally in a variety of tissues. The 5-HT_{1C} designation is vacant, as the receptor was renamed 5-HT_{2C}, due to structural, operational, and transductional similarities with the 5-HT₂ receptor family.

Serotonergic System. Table 3 5-HT₅, 6, 7 receptors

Nomenclature	5-HT _{5A}	5-HT _{5B}	5-HT ₆	5-HT ₇
Selective Agonist	–	–	–	–
Rank order				5-CT > 5-HT > 8-OH-DPAT > sumatriptan
Selective antagonists (pK_B)	A-843277 (8.1)	–	Ro 630563 (7.9)	SB 258719 (7.9)
	A-833551 (8.5)		SB 271046 (7.8)	SB 269970 (9.0)
			SB 357134 (8.5)	
Radioligands	[¹²⁵ I]LSD	[¹²⁵ I]LSD	[¹²⁵ I]SB 258585	[¹²⁵ I]LSD
	[³ H]5-CT	[³ H]5-CT	[¹²⁵ I]LSD	[³ H]SB 269970
			[³ H]5-HT	[³ H]5-CT
				[³ H]5-HT
G protein effector	G _s Gi? increases (decreases) cAMP formation	not identified	G _s preferentially increases cAMP formation	G _s preferentially increases cAMP formation
Localisation	CNS: Hippocampus (CA1, CA3, DG), cortex, cerebellum (granular layer), olfactory bulb, habenula, spinal cord		CNS: Caudate putamen, olfactory tubercle, nucleus accumbens, cortex, hippocampus (CA1, CA3, DG)	CNS: Hippocampus (CA1, CA2), hypothalamus, thalamus, superior colliculus, raphe nuclei
			Peripheral: Superior cervical ganglion	Peripheral: GI and vascular smooth muscle, sympathetic ganglia
	Peripheral: None identified			

**Serotonergic System. Figure 1** Graphical representation of the current classification of 5-hydroxytryptamine (5-HT) receptors. Receptor subtypes represented by shaded boxes and lower case designate receptors that have not been demonstrated to definitively function in native systems. Abbreviations: 3'-5' cyclic adenosine monophosphate (cAMP); phospholipase C (PLC); negative (–ve); positive (+ve)

5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors exhibit 46–50% overall sequence identity and couple preferentially to G_{q/11} to increase inositol phosphates and cytosolic [Ca²⁺] (see Tables 1–3).

5-HT₃ receptors belong to the ligand-gated ion channel receptor superfamily, similar to the nicotinic acetylcholine or GABA_A receptors and share electrophysiological and structural patterns. The receptors

are found on central and peripheral neurons where they trigger rapid depolarization due to the opening of nonselective cation channels (Na^+ , Ca^{2+} influx, K^+ efflux). The response desensitizes and resensitizes rapidly. The native 5-HT_3 receptor, as revealed by electron microscopy in neuroblastoma-glioma cells, is a pentamer, and there may exist three different subunits, 5-HT_{3A} , 5-HT_{3B} , and 5-HT_{3C} . Currently, the consensus is that native receptors are best reproduced by the heteromeric combination of 5-HT_{3A} and 5-HT_{3B} subunits. 5-HT_{3B} homomers lack functions (as is the case for 5-HT_{3C}), and it appears that 5-HT_{3A} receptors need to form heteromers to become functional (with, e.g. 5-HT_{3B}). It has been suggested that 5-HT_3 receptors can heterodimerize with other members of the superfamily (e.g. nicotinic $\alpha 7$), but this has not been demonstrated *in situ*.

5-HT_4 , 5-HT_6 , and 5-HT_7 receptors all couple preferentially to G_s and promote cAMP formation, yet they are regarded as distinct receptor classes because of their limited (<35%) overall sequence identities. This subdivision is arbitrary and may be subject to future modification.

Two subtypes of the 5-HT_5 receptor (5-ht_{5A} and 5-ht_{5B}), sharing 70% overall sequence identity, have been found in rodents. The human 5-ht_{5B} receptor gene does not encode a functional protein due to the presence of stop codons in its coding sequence. Human recombinant 5-ht_{5A} receptors inhibit forskolin-stimulated cAMP production, although the receptor may also couple positively to cAMP. Currently, a physiological readout for this receptor is still missing, although A-843277, a selective 5-ht_{5A} antagonist has antidepressant/antipsychotic properties in rodent models.

Additional endogenous 5-HT receptors have been defined pharmacologically, although a corresponding gene product encoding the receptor has not yet been identified. As long as their structure is unknown, these receptors are regarded as orphans in the current nomenclature. One of these however, the so-called “ 5-HT_1 -like” receptor mediating direct vasorelaxation corresponds to the 5-HT_7 receptor. On the other hand, the situation with the remaining **▶ orphan receptors** (see 3) has not evolved further and thus the status quo ante remains. In particular, no progress has been made with the so-called 5-HT_{1P} receptor, which is present in the gut and whose pharmacology is reminiscent of the 5-HT_4 receptors, with the restriction that some of the ligands described, like the 5-HT dipeptides do not affect 5-HT_4 receptors. The 5-HT_{1P} could be a heterodimer, but the putative partners remain to be identified.

Pathophysiology/Clinical Applications

5-HT has been implicated in the etiology of numerous disease states, including depression, anxiety, social phobia, schizophrenia, obsessive compulsive disorders,

panic and sleep disorders, migraine, vascular and pulmonary hypertension, eating disorders, nausea/vomiting, **▶ irritable bowel syndrome** (IBS) and other functional GI disorders, by interacting at different receptors or transporters (see 2, 3, 5, 6).

Indeed, 5-HT is also a substrate for the 5-HT transporter, itself an important player in the treatment of depression, and more recently for the whole range of anxiety disorders spectrum (GAD, OCD, social and other phobias, panic and post-traumatic stress disorders). It is the target for **▶ SSRIs** (selective serotonin reuptake inhibitors) such as fluoxetine, paroxetine, fluvoxamine, and citalopram or the more recent dual reuptake inhibitors (for 5-HT and noradrenaline, also known as SNRIs) such as venlafaxine. Currently, there are efforts to develop triple uptake inhibitors (5-HT , NE, and DA). Further combinations are possible, e.g. SB-649915, a combined 5-HT_{1A} , 5-HT_{1B} , 5-HT_{1D} inhibitor/selective serotonin reuptake inhibitor (SSRI), is investigated for the treatment of major depressive disorder.

5-HT_{1A} receptor agonists, such as buspirone or gepirone, are being used/developed for the treatment of anxiety and depression. Furthermore, the 5HT_{1A} receptor and β -adrenoceptor antagonist, pindolol, was reported to enhance the therapeutic efficacy and shorten the onset of action of SSRIs upon co-administration in severely depressed patients. Both positive and negative findings have been reported, as is common in depression trials; it is suggested that the combination therapy functions provided that sufficient 5-HT_{1A} receptor occupancy is reached. Flesinoxan, a 5-HT_{1A} receptor agonist, was initially developed as an anti-hypertensive agent, however its effects in patients were disappointing and this approach has now been abandoned. S-37245 is a 5-HT_{1A} antagonist which is under investigation for the potential treatment of anxiety and depression.

Interest in 5-HT_{1B} receptor agonists has been triggered by the anti-migraine properties of sumatriptan, a non-selective $5\text{-HT}_{1D/1B}$ receptor agonist and many others (dihydroergotamine (DHE), zolmitriptan, naratriptan, rizatriptan, eliotriptan, almotriptan, donitriptan, etc). The development of 5-HT_{1B} agonist “serenics” such as eltoprazine was not successful; the expected anti-aggressive effects were not observed in patients. AZD-8129 a 5-HT_{1B} was recently discontinued in anxiety/depression.

Sumatriptan and Naratriptan also bind to 5-HT_{1F} receptor. It has been hypothesized that they might be a target for antimigraine drugs. 5-HT_{1F} receptor mRNA has been detected in the trigeminal ganglia, stimulation of which leads to plasma extravasation in the dura, a component of neurogenic inflammation thought to be a possible cause of migraine. LY 334370, a selective 5-HT_{1F} receptor agonist, inhibits trigeminal stimulation-induced early activated gene expression in nociceptive

neurons in the rat brainstem. LY-349950 was in clinical development for migraine, but was discontinued.

Ketanserin a selective 5-HT_{2A} antagonist was developed for the treatment of hypertension, but 5-HT_{2A} receptor antagonism as an antihypertensive principle has been abandoned, since ketanserin is a potent α_1 adrenoceptor antagonist. LSD and other hallucinogens most probably produce hallucinations by activating 5-HT_{2A} receptors. Although their selectivity vis-a-vis 5-HT_{2B} and 5-HT_{2C} receptors is rather limited, this represents currently the best possible explanation. 5-HT_{2A} receptor antagonists such as risperidone, ritanserin, seroquel, olanzapine, or MDL 100907 have been indicated/developed for the treatment of schizophrenia. However, development of MDL 100907 for acute schizophrenia was stopped. The combination of dopamine D₂ and 5-HT_{2A} receptor antagonism may still explain the beneficial antipsychotic activity of drugs such as clozapine, olanzapine, seroquel, and others.

BW 723C86 has agonist selectivity at the rat 5-HT_{2B} receptor, although less marked at human receptors, but activation of the 5-HT_{2B} receptor is most probably responsible for the valvulopathies reported for appetite suppressant preparations containing dex-fenfluramine, and 5-HT_{2B} agonism is therefore not being pursued anymore. 5-HT_{2B} receptor antagonists such as SB 200646 may be indicated for the treatment of migraine prophylaxis, given the vasodilatory role of this receptor and that a number of "older" antimigraine drugs share 5-HT_{2B} receptor antagonism. 5HT_{2B} antagonism may be a viable principle in pulmonary hypertension. On the other hand, PGN-1164 a 5-HT_{2B} receptor antagonist was in clinical trials for IBS, but stopped for PK reasons.

The anxiogenic component of mCPP may be mediated by 5-HT_{2C} receptor activation, and selective 5-HT_{2C} receptor antagonists such as SB 242084 or agomelatine display anxiolytic/antidepressant properties in animal models. Central 5-HT_{2C} receptor activation produces a tonic, inhibitory influence upon frontocortical dopaminergic and adrenergic, but not serotonergic transmission. 5-HT_{2C} receptor knockout mice have spontaneous convulsions, cognitive impairment, increased food intake, and obesity, but similar effects are not reproduced by selective antagonists, suggesting that these changes may result in part from neuroadaptation. Agomelatine a 5-HT_{2C} antagonist, combined with melatonin 1/2 receptor agonist, is effective in several animal models of depression and anxiety; it increases prefrontal dopamine and noradrenaline release. Several large, double blind placebo-controlled studies of Agomelatine have demonstrated that it is a clinically effective and well-tolerated antidepressant with a very low incidence of side effects. 5-HT_{2C} agonists (e.g. Org 37684, Ro 60-0175, YM348, or WAY-163909) produce positive effects in rodent models of depression, panic, and compulsive disorders.

On the other hand, 5-HT_{2C} agonists are being investigated in obesity, depression, and schizophrenia, and SCA-136, has entered phase II trials in schizophrenia.

The 5-HT₃ receptor antagonists ondansetron, granisetron, and tropisetron are used clinically in chemotherapy- and radiotherapy-induced nausea and vomiting. Since 5-HT₃ receptor activation in the brain leads to dopamine release, and 5-HT₃ receptor antagonists produce central effects compared to those of antipsychotics and anxiolytics, schizophrenia and anxiety were considered as potential indications. 5-HT₃ receptor antagonists have been reported to induce cognition enhancing effects. However, there are not enough clinical data to substantiate such activities. Similarly, that 5-HT₃ antagonists should prove useful in the treatment of migraine did not materialize in clinical studies. E-3620, a dual 5-HT₃ antagonist and 5-HT₄ agonist activity, was under development for IBS, chemotherapy-induced emesis, gastro-esophageal reflux but has been stopped. On the other hand, Alosetron (Lotronex[®]) is now indicated for women suffering from ►IBS with diarrhea (IBS-D).

Selective 5-HT₄ receptor ligands may have therapeutic utility in a number of disorders, including cardiac arrhythmia, neuro-degenerative diseases, depression, urinary incontinence, and GI motility disorders. Cisapride, a gastroprokinetic agent, acts as an agonist at the 5-HT₄ receptor, but was withdrawn due to side effects (QT prolongation). Metoclopramide is a 5-HT₄ agonist, but carries dopaminergic side effects. Other 5-HT₄ agonists in development are mosapride and prucalopride: the latter has been shown to increase bowel movement in volunteers, whereas mosapride had positive effects in functional dyspepsia. Piboserod (SB 207266), a potent and selective 5-HT₄ antagonist was investigated in IBS-D and increases oro-caecal transit time. Tegaserod (Zelmac/Zelnorm[®]), a new generation 5-HT₄ receptor partial agonist, was recently introduced in various markets to treat constipation predominant IBS (IBS-C) and constipation. Tegaserod's therapeutic activity in functional motility disorders of the upper G.I. tract is under clinical investigation.

Antipsychotics (clozapine, olanzapine, fluperlapine, and seroquel) and antidepressants (clomipramine, amitriptyline, doxepin, and nortriptyline) act as 5-HT₆ receptor antagonists. This attribute tempted speculations of an involvement of the 5-HT₆ receptor in psychiatric disorders, although these drugs are by no means selective. Selective 5-HT₆ receptor antagonists (e.g. Ro 04-6790, SB-399885, and BGC20-760) have positive effects in preclinical models of memory impairment and cognition, presumably by modulating Ach release. Clinical developments in 5-HT₆ antagonist field therefore concentrates on Alzheimer's disease. On the other hand, 5-HT₆ receptor agonists (e.g. WAY466 or LY586713) presumably by modulating GABA and BDNF have been shown to produce positive effects in preclinical

models for anxiety and depression. WAY-181187, a 5-HT₆ agonist, is being developed for generalized and acute anxiety disorders.

Atypical antipsychotics, e.g., clozapine, risperidone and antidepressants also have high affinity for the 5-HT₇ receptor. 5-HT₇ receptor downregulation occurs after chronic antidepressant treatment, and acute, but not chronic, stress regulates 5-HT₇ receptor mRNA expression. The presence of 5-HT₇ sites in the limbic system and thalamocortical regions, suggests a role in affective disorders, and the expression in the supra chiasmatic nucleus suggests a role in circadian rhythms. Various 5-HT₇ antagonists (e.g. SB-258741) were/are investigated in psychosis, schizophrenia, and circadian disorders.

- ▶ Antidepressant Drugs
- ▶ Antipsychotic Drugs
- ▶ Emesis

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Serpentine Receptors

- ▶ G-protein-coupled Receptors

Serum Sickness

Serum sickness is an inflammatory condition caused by the deposition of immune complexes in blood vessel walls and tissues.

- ▶ Allergy
- ▶ Humanized Monoclonal Antibodies

Seven Transmembrane Helix Receptors

- ▶ G-protein-coupled Receptors

Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

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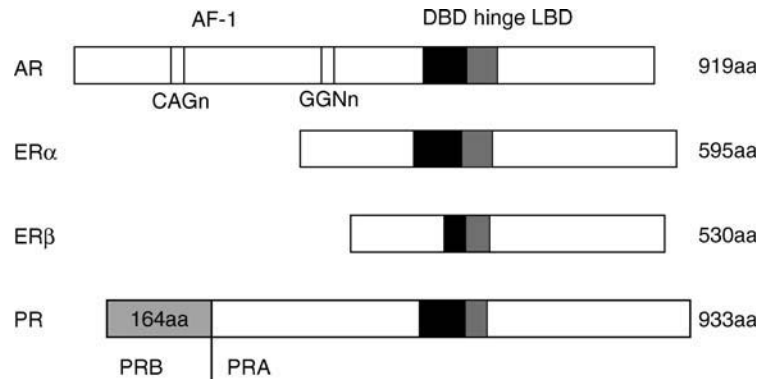
Definition

Sex steroid receptors are members of the steroid hormone receptor (SHR) family that ligand-dependently regulate functions of the sexual organs. Sex steroid receptors are the androgen receptor [1] (AR), the estrogen receptor α [2] and β [3] (ER α , ER β), and the progesterone receptor [4] (PR).

Basic Characteristics

The subgroup of SHRs belongs to the superfamily of ▶ **nuclear receptors** (NRs), which transactivate target genes ligand-dependently. Unliganded SHRs are associated with large multiprotein complexes of ▶ **chaperones** in the cytoplasm, in contrast to other NRs. SHRs comprise the glucocorticoid receptor and the mineralocorticoid receptor and the sex ▶ **steroid receptors**.

SHRs are built in a modular structure with similar structure elements. They contain a DNA-binding domain (DBD), a hinge region with a nuclear location signal (NLS), a ligand-binding domain (LBD) and several transcriptional activation functions (Fig. 1).



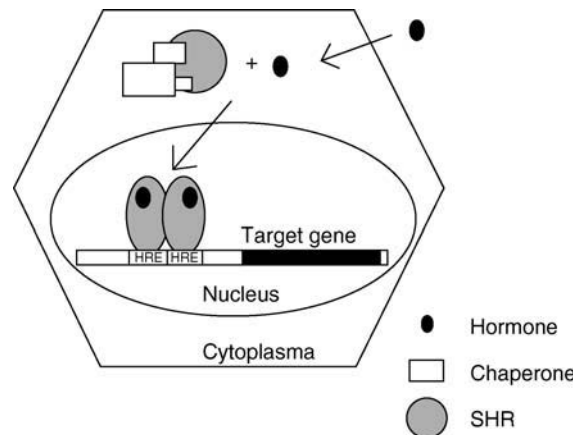
Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor.
Figure 1 Schematic structures of AR, ER α , ER β , and PR.

Their ligands are fat-soluble steroid hormones derived from cholesterol that bind to the LBD of their specific intracellular SHR after diffusing into the cell. After binding of the steroid hormone (K_d between 0.1 and 4 nM) the conformation of the SHR changes, exposing the NLS and the complex of steroid hormone and SHR gains access to the nucleus (Fig. 2). Utilizing the two zinc fingers of their DBD, SHRs bind as homodimers to unique DNA sequences called hormone response elements (HREs). The HRE is comprised of two half-sites organized as palindrome with a three nucleotide spacer. SHRs regulate the expression of target genes after association with large multisubunit complexes that contain [transcriptional co-activators](#) such as histone acetylases and several other proteins that facilitate transcription. Several signalling pathways furthermore influence the activity of SHRs, by modifying either SHRs directly or partner proteins. SHRs can also act without binding to DNA via interaction with other transcription factors, thereby altering their own or their partner's properties.

The physiological and pathophysiological roles of the sex steroid receptors are diverse and will be summarized separately for AR, ER α , ER β s, and PR in the following paragraphs. Estrogen related receptors (ERRs) share structural and functional similarities with ERs. They are orphan receptors indicating that there is no known natural [ligand](#) and are therefore not grouped as SHR.

Androgen Receptor

Androgens act via the AR and play an important role in the development and differentiation of the male sexual organ. Furthermore, they are involved in several diseases, the most important being partial and complete androgen insensitivity syndrome (AIS; formerly known as the testicular feminization syndrome), spinal and bulbar muscle atrophy (SBMA; Kennedy's disease), and the neoplastic transformation of the prostate. The two natural occurring androgens are testosterone (T) and the



Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor.

Figure 2 The classical activation pathway of SHR. SHRs (gray circle) are associated with chaperones (rectangles). After binding of steroid hormones (black circle) SHRs activate target genes in the nucleus. Additional regulation mechanisms, e.g., phosphorylation are described in the text.

more potent 5 α -dihydrotestosterone (DHT). T is mainly produced by the Leydig cells of the testis and can also be produced in most peripheral tissues from the adrenal-produced inactive steroid precursors dehydroepiandrosterone, its sulfate, and androstenedione. T is converted into DHT by the 5 α -reductase enzyme expressed in the urogenital tract. Besides positive regulation of target genes by the androgen-loaded AR, there is growing evidence of additional regulation pathways and indirect regulation mechanism of the AR. The expression of specific transcriptional coactivators of the AR in different tissues can fine-tune the transcriptional AR-activity. Ligand-independent activation of the AR by protein kinase pathways can circumvent the need for androgens. In addition, protein–protein interactions of the AR with

other transcription factors regulate the transcriptional activity of these partner proteins.

Eight exons of the AR gene encode a protein of around 917 aa depending on two polymorphic regions of polyglutamines (CAG) and polyglycines (GGN) in the N-terminal activation domain. Two isoforms are detected in tissues: the predominant (80%) 110 kD (B isoform) and 87 kD (A isoform). It is not clear whether the two isoforms also serve different functions.

The structure of the AR comprises an N-terminal transactivation domain of around 500 aa, a DBD of 66–68 aa, and a LBD of 250 aa. The hinge region contains the lysine-rich NLS. The AR possesses two activation functions (AFs): AF-1 in the N-terminal region and the AF-2 core domain in the LBD.

The AF-1 contains two polymorphic regions of CAG and GGN repeats. Normally the number of the 5' CAG repeats is 11–31 (average 21) whereas up to 50 repeats are found in individuals affected with SBMA. Since the number of glutamines inversely correlates with the transcriptional activity of the AR these amplified repeats lead to a reduced activity of the AR. The increased size of a polymorphic tandem CAG repeat is associated with the X-linked spinal and bulbar muscular atrophy and may also be associated with oligospermic infertility in men and with low serum androgens in a subset of anovulatory female patients. On the other hand, a shorter CAG length correlates with a higher risk; more severe and earlier onset of prostate cancer probably resulting from a higher activity of the AR. Experimental evidence also correlates increased or prolonged induction of AR activation with a higher incidence or acceleration of prostate cancer.

In the beginning, prostate cancer cells are largely dependent on androgens for growth and survival. Observations, that castration is beneficial in prostate cancer made androgen ablation and antiandrogen therapy a standard treatment for patients with metastatic prostate cancer following surgery of the tumor tissue. Antiandrogen therapy includes inhibition of androgen synthesis by aminoglutethimide or ketoconazole, inhibition of **►5 α -reductase** by finasteride in combination with AR **►antagonists** such as flutamide, or cyproterone acetate.

Unfortunately, remaining prostate cancer cells eventually adapt to grow in the low-androgen environment, rendering the tumor growth independent of androgens. Androgen-independent prostate cancer may result from one or more of the following mechanism: increased gene copy number, altered interaction of the AR with coregulatory proteins, e.g., resulting from AR mutations, bypassing of the AR pathway, or ligand-independent activation of AR, e.g., by protein kinase pathways.

Estrogen Receptor

Estrogens mainly affect the growth and maturation of the female reproductive system and the maintenance

of its reproductive capacity [5]. In addition, estrogens act on several other tissues, e.g., on lipid and bone metabolism. Uterus, placenta, and testis are the principal sites of 17 β -estradiol (E2) production. **►Agonists** of ERs are used for treatment of menopausal symptoms **►hormone replacement therapy** (HRT), osteoporosis and cardiovascular diseases. **►Antagonists** of ERs such as tamoxifen are used for treatment of breast cancer. Pure antagonist of ER that are as effective as tamoxifen without having tamoxifen's partial agonistic effects on ERs are in different phases of testing. The pure antagonist fulvestrant (ICI 183,780) is licensed as treatment for advanced breast cancer.

The main isoforms of the human ERs are ER α and ER β , which display distinct expression patterns. Additional ER isoforms, generated by alternative mRNA splicing, have been identified in several tissues. A cell-specific localization for each of the ER subtypes is found in the majority of the reproductive organs studied. The role of the different ER isoforms in modulating the estrogen response or in tumorigenesis is not completely understood. In addition, ERRs where coexpressed with ERs can influence the expression level of ER target genes either directly or by interaction with ERs. ERs bind most ligands with similar affinities and display equal transcriptional activation. However, in some assays ER isoforms respond differently to ligands. The naturally occurring phytoestrogen genistein or antiestrogens, such as tamoxifen or raloxifen are examples of these selective ERs modulators (**►SERMs**). The characterization of SERMs that specifically regulate defined functions promises to increase efficacy and reduce side effects in estrogen-regulated processes (Table 1).

ER α

ER α (also called ESR1 and ESRA) is involved in the differentiation and maintenance of reproductive, neural, skeletal, and cardiovascular tissues. Two separate AFs mediate transcriptional activation, the ligand-dependent AF-2 in the LBD, and the ligand-independent AF-1 in the N-terminus. After binding of estrogen to the LBD, ER α activates target genes such as the progesterone receptor gene by binding to the estrogen response elements (EREs). Besides this classical activation, nonestrogen-dependent activation of ER α has been described. Signaling pathways and extracellular signals such as EGF, IGF-I, or insulin, stimulate phosphorylation of the receptor. Phosphorylation of ER α affects all steps of transcriptional activation, such as ligand binding, dimerization, DNA binding and interaction with cofactors. Heregulin is an example of an extracellular signal modulating ER activity. After binding to its receptor HER-2 ER is rapidly phosphorylated on tyrosine residues, followed by transcription of the *PR* gene. Since heregulin promotes hormone-independent

Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor. Table 1 Basic characteristics of sex steroid receptors

SHR	Gene map locus	cDNA size	Natural agonist	Binding half-site	Main expression
AR	Xq11–q12	ca. 919 aa (see text)	5 α -Dihydrotestosterone (DHT), testosterone (T)	AGAACA	Prostate, male urogenital system, muscle
ER α	6q25.1	595 aa	17 β -Estradiol (E2)	AGGTCA	Ovary, uterus, mammary gland, vagina, testis (Leydig cells), bone
ER β	14q22–q24	530 aa, 583 aa, further isoforms	17 β -Estradiol (E2)	AGGTCA	Ovary, testis (Sertoli and Leydig cells, efferent ducts), prostate, bone, thymus, spleen, brain
PR	11q22	Two isoforms: PRA 769 aa, PRB 933 aa	Progesterone	AGAACA	Uterus, ovary, central nervous system

growth of breast cancer cells, activation of ER by heregulin or HER-2 may be involved in the development of E2-independent cancer cells growth.

ER α can also regulate gene expression by interacting with different transcription factors. For example, interaction of ER α with the c-Rel subunit of NF- κ B prevents binding to NF- κ B response element resulting in reduced interleukin-6 transcription. Here, ER α acts E2-dependently but without directly binding to DNA. However, the complex formation of ER with the transcription factor Sp1 is hormone-independent and enhances Sp1 binding to DNA. ER α as well as ER β thereby enhance transcription of the retinoic acid receptor *al* gene. Other partner proteins are fos/jun family members, which regulate gene expression via AP-1 sites. In this situation E2 can either act as agonist in the presence of ER α or as antagonist in the presence of ER β . Another possibility to modulate ER signaling is the aryl hydrocarbon receptor (AhR). Ligands for AhR mediate antiestrogenic effects by several pathways.

The measurement of ER has become a standard assay in the clinical management of breast cancer. The presence of ER α identifies those breast cancer patients with a lower risk of relapse and better clinical outcome. Receptor status also provides a guideline for those tumors that may be responsive to hormonal intervention. But only about half of ER-positive patients respond to hormonal therapies. Of those who respond initially, most will eventually develop an estrogen unresponsive disease following a period of treatment even though ER α is often still present. Mutant receptors and constitutively active receptors as well as hormone-independent activation of the ER α are discussed. The involvement of ER β isoforms is under investigation.

Analysis of ER α -deficient mice showed that both sexes are infertile and display a variety of phenotypic changes associated with the gonads, mammary glands, reproductive tracts, and skeletal tissues. In addition,

both hyperplasia and hypertrophy of adipocytes were found in these mice.

In females, ovariectomy or menopause leads to rapid loss of trabecula bone and the osteoporosis indicating that E2 maintains bone mass. E2 enhances bone formation by osteoblasts and suppresses bone resorption by osteoclasts by regulating several important growth factors. In mice, ER α seems to induce growth but not maintenance of trabecular bone whereas ER β terminates growth during late puberty. However, while in humans the role of ER α and ER β in bone is not completely clear, ESRA polymorphism is related to bone density and height during late puberty and at attainment of peak bone density in young men.

ER β

In 1995 the discovery of ER β (also called ESR2 and ESRB) explained many actions of estrogens in tissues where no ER α was found. ER β shows high homology to ER α in the DBD and LBD, but encodes a distinct transcriptional AF-1 domain. At least five isoforms, designated ER β -1 through ER β -5, are described that differ in their C-terminal sequences and tissue expression patterns, or have extended N-termini. This new complexity of isoforms is further enhanced by the fact that ER β isoforms cannot only heterodimerize with each other but also with ER α . The functional consequences for the action of estrogens depending on the expression pattern are only beginning to be evaluated.

ER β is highly expressed in ovary, male organs, and parts of the central nervous system (CNS), but also in other organs such as spleen and thymus. The phytoestrogen genistein binds better to ER β than to ER α , whereas the partial ER α agonists tamoxifen, raloxifen and ICI-164 384 are antagonists for ER β . It has been postulated that co-factor recruitment is different for the ERs, however knowledge about this interesting field of selective ER regulation is only beginning to accumulate. Since only ER β is expressed widely in the male

urogenital tract of several animals it is now under evaluation whether the pronounced effects of E2 in men are caused by direct action of E2 on ER β in these reproductive organs. The view that E2 acts only indirectly by reducing androgen levels via the CNS clearly has to be corrected.

Analysis of ESRB^{-/-} mice showed fewer and smaller litters than wild type mice as well as abnormal vascular function and hypertension. The reduction in fertility was attributed to reduced ovarian efficiency. Mutant females had normal breast development and lactated normally. Older mutant males displayed signs of prostate and bladder hyperplasia. *Esr2*-deficient mice furthermore display diverse regulatory defects in the function of brain, lung, and white blood cells. The results indicated that ESRB is essential for normal ovulation efficiency but is not essential for lactation, female or male sexual differentiation, or fertility.

Progesterone Receptor

The PR is involved in diverse functions in female reproduction, such as implantation of the embryo, and in the maintenance of pregnancy. Progesterone is mainly produced in the corpus luteum in the second half of the menstrual cycle and in early pregnancy, later in the placenta. The PR is expressed in the uterus, ovary, and the CNS. In men there is no known function. Estrogens induce expression of the *PR* gene. PR agonists such as medroxyprogesterone or the synthetic R5020 are called progestins or gestagens.

The human PR exists as two functionally distinct isoforms PRA and PRB transcribed from two promoters from a single gene. PRA lacks the N-terminal 164 aa and is a 769 aa protein. PRB functions as a transcriptional activator in most cell and promoter contexts. In contrast, PRA is transcriptionally inactive and functions as a strong ligand-dependent transdominant repressor of SHR transcriptional activity. Different cofactor interactions were demonstrated for PRA and PRB, probably due to an inhibitory domain within the first 140 aa of PRA, which is masked in PRB. Both PR isoforms however, repress estradiol-induced ER activity when liganded. Several other mRNA isoforms are present in PR-positive tissues such as breast cancer with unknown clinical significance.

PRs also interact with other signaling pathways, which can, e.g., be regulated by phosphorylation. Independent of transcriptional activation of PR, progestins can activate cytoplasmic signaling molecules including SRC and downstream MAP kinase in mammalian cells via interaction by a specific polyproline motif in the N-terminal domain of PR.

Mice models reveal that both PR forms are physiologically important. Mice lacking the *PR* gene fail to ovulate, are infertile, and have impaired thymic function. Selective PRA-deficient female mice are infertile due to reduced oocyte and uterine deficiency

in implantation. However, these mice had normal mammary epithelium proliferation and differentiation and showed normal thymic involution. In mice, PR regulates expression of proteases that degrade the follicular wall thereby facilitating ovulation.

In breast cancer patients, total PR status is measured for hormonal treatment. The presence of PR is associated with increased survival rates and hormonal responsiveness of mammary tumors. PR agonists are widely used in contraception, HRT, breast cancer, and endometrial hyperplasia. ▶ **Antiprogestins** such as RU486 are used for blocking ovulation and preventing implantation, and in addition they are in clinical testing for the induction of labor and to control various neoplastic transformations.

▶ **Selective progesterone receptor modulators (SPRM)** (mesoproggestins) are PR ligands with agonistic and antagonistic activities. Some SPRM show weak antigluccorticoid or mixed androgenic/antiandrogenic activities. SPRM are currently tested and may be useful, e.g., for the treatment of endometriosis.

Drugs

In clinical use are pure and partial agonists and antagonists (see individual SHR) as contraceptives, treatment for hormonal ablation in breast and prostate cancer, and HRT in osteoporosis.

▶ **Contraceptives**

▶ **Gluco-mineralocorticoid Receptors**

▶ **Selective Sex Steroid Receptor Modulators**

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SGLT

▶ **Na⁺-dependent Glucose Cotransporter**

SH2, Domains

The Src homology 2 domain (or SH2-domain) is a protein domain of about 100 amino acid residues first identified in the tyrosine kinase Src. SH2-domain

mediates protein–protein interaction with a phosphorylated tyrosine residue in interacting proteins. This binding triggers a change in sub-cellular localization or enzyme activity crucial in signalling pathway.

- [Phospholipids Glossary](#)
- [Adaptor Proteins](#)
- [Tyrosine Kinases](#)

SH3 Domain

Synonyms

src homology 3

Definition

Protein-protein interaction domain that binds to poly-proline motifs with the sequence PXXP. Particularly important in assembling protein complexes at activated receptors which contain intrinsic tyrosine kinases.

- [Adaptor Proteins](#)

Shaker-Channels

Shaker-channels, eag (ether-á-go-go)-channels, slo (slow-poke)-channels were cloned from behavioral *Drosophila melanogaster* mutants. The channels were named according to the *Drosophila* mutant phenotype, Shaker, ether-go-go, slow-poke. Subsequently, eag-cDNA was used to clone related voltage-gated potassium channel subunits erg (eag-related) and elk (eag-like). The human erg ortholog (HERG) mediates cardiac IKS.

- [Voltage-gated K⁺ Channels](#)

Shine-Dalgarno Interaction

The Shine-Salgano interaction is a base pairing interaction that occurs during translation initiation in prokaryotes between the Shine-Dalgarno sequence on messenger RNA (mRNA) and the anti-Shine-Dalgarno

sequence on 16S ribosomal RNA (rRNA). The Shine-Dalgarno sequence is a purine-rich sequence located four to seven bases upstream of the initiation codon in prokaryotic mRNAs. It has variable length and complementarity to the anti-Shine-Dalgarno sequence found in the 3'-end region of all bacterial 16S rRNAs (and in archaeal and chloroplast 16S rRNAs).

- [Ribosomal Protein Synthesis Inhibitors](#)

SIADH

Syndrome of inappropriate antidiuretic hormone is defined by water retention, dilutional hyponatraemia and decreased volume of highly concentrated urine. There are several causes which can result in SIADH, neoplasms ectopic secreting AVP, ectopic release of AVP by various diseases or drugs, exogenous administration of AVP, desmopressin, lysipressin or large doses of OT (iatrogenic SIADH).

- [Vasopressin/Oxytocin](#)

Sialic Acid

Sialic acid is a carbohydrate that can be attached to certain molecules, e.g., epoetin alfa.

- [Hematopoietic Growth Factors](#)

Sialin

Sialin was first identified as the product of the gene defective in sialidosis, a lysosomal storage disorder. The transporter mediates the movement of sialic acid out of lysosomes by coupling to the proton electrochemical gradient across the lysosomal membrane. Unlike the vesicular neurotransmitter transporters which are antiporters, sialin is a symporter with sialic acid and protons both moving out of the lysosome.

Signal Peptidases

Specific proteases located on the luminal side of the endoplasmic reticulum. They cleave the amino-terminal peptides from the precursor forms of membrane and secretory proteins.

► Protein Trafficking and Quality Control

Signal Recognition Particle

The signal recognition particle (SRP) is a cytosolic ribonucleoprotein complex which binds to signal sequences of nascent membrane and secretory proteins emerging from ribosomes. The SRP consists of a 7S RNA and at least six polypeptide subunits (relative molecular masses 9, 14, 19, 54, 68, and 72 kD). It induces an elongation arrest until the nascent chain/ribosome/SRP complex reaches the translocon at the endoplasmic reticulum (ER) membrane.

► Protein Trafficking

Signal Transducer and Activator of Transcription

Definition

STAT.

► JAK-STAT Pathway

Simple Diffusion

Permeation of a drug through biological membranes according to the electrochemical gradient. This type of drug transport can be explained by the pH-partition theory.

► Drug Interaction

Simulated Annealing

Simulated annealing is a type of molecular dynamics experiment in which the temperature of the system is cycled over time with the goal of widely sampling conformational space. There are two basic ideas. The first is to create a computational analog of experimental annealing techniques and the second is to use controlled mechanisms for obtaining different initial structures by using temperature to surmount torsional barriers. Heating to a higher temperature (e.g., 1,000K) allows the system to rearrange from the present state, cooling to a lower temperature brings the system into a stable state. The cycle is repeated several times (e.g., 100), so that multiple conformations may be obtained. Favorable and stable geometries occur in clusters of similar conformations. During the cooling phases it is possible to introduce constraints between atoms coming from experiments. This procedure is applied to calculate structures based on atom distances like nuclear Overhauser effect (NOE) distances and other geometrical data produced by nuclear magnetic resonance (NMR) spectroscopy.

► Molecular Modeling

► NMR-based Ligand Screening

Single Nucleotide Polymorphism

Single Nucleotide Polymorphisms (SNPs) are single base pair positions in genomic DNA at which normal individuals in a given population show different sequence alternatives (alleles) with the least frequent allele having an abundance of 1% or greater. SNPs occur once every 100–300 bases and are hence the most common genetic variations.

► Bioinformatics

► Pharmacogenomics

Sinus Rhythm

The sinus rhythm is the heart rhythm in which the sinus node generates an electrical impulse that travels through specialized cells (that form a conduction system) and leads to a ventricular contraction.

- Cardiac Glycosides
- Antiarrhythmic Drugs

siRNAs

Small interfering RNAs (siRNA) are the mediators of gene-specific silencing by RNA interference. SiRNA stands for small interfering RNA duplexes. They are typically 21-23 bp in length. SiRNA were either chemically synthesised for experimental purposes or produced by Dicer-mediated cleavage of long double-stranded RNA.

- Antisense Oligonucleotides

Skeletal Metabolism/Turnover

- Bone Metabolism

Skinned Fiber

A skinned fiber is a muscle fiber, the sarcolemma of which has been mechanically removed or which is made freely permeable to small molecules, such as Ca^{2+} , Mg^{2+} , EGTA, ATP, soluble enzymes and others by a chemical agent (saponin, β -escin or Staphylococcus α -toxin). The organization of the sarcoplasmic reticulum (SR) and myofibrils is kept as they are in the living muscle.

- Ryanodine Receptor

Skp1/Cul1/F-Box Protein Complex

Synonyms
SCF

Definition

The SCF, a ubiquitin ligase complex, consists of the primary subunits Skp1, Cullin and Rbx/Roc1. While the Rbx/Cul components form the E3 ligase catalytic core

which associates with the Ubiquitin conjugating enzyme (E2), specificity towards substrates is mediated by the interaction with a large set of so called F-box proteins which directly recruit the target proteins for ubiquitination.

- Ubiquitin/Proteasome

Sleep

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Definition

Sleep is a state common to most mammals, birds, fishes and invertebrates. It describes a state in which brain activity changes from that of the waking brain, resulting in outward signs such as immobility and a decreased responsiveness to external stimuli. The function of sleep is not yet fully understood, although its importance is undeniable, in that an organism chronically deprived of sleep will eventually die. Sleep itself is not homogenous, and can be divided into various states representing different patterns of electrical activity. The underlying biology and complex interaction of various neurotransmitter systems in initiating, maintaining and shaping sleep are now beginning to be better understood, paving the way for more precise and effective pharmacological treatment of sleep disorders.

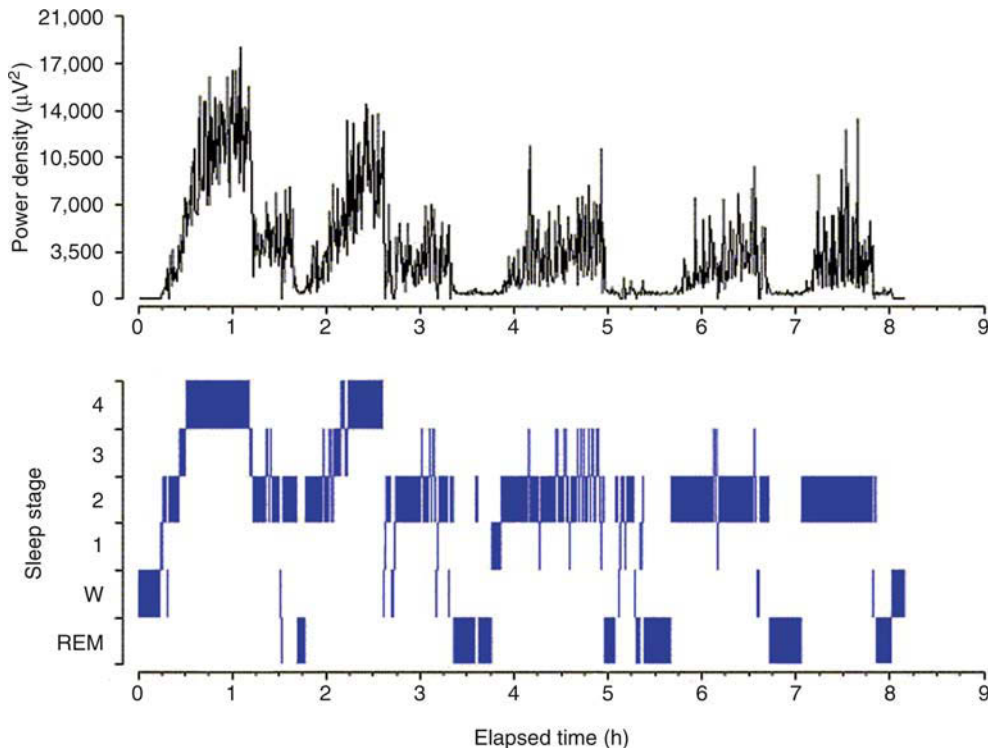
Basic Mechanisms

Molecular Mechanisms Underlying Sleep: Focus on GABA

Like waking, sleep is an active state of the brain. During this heterogeneous and rapidly changing state several restorative functions take place, although the neural substrates of somatic and cognitive restoration remain elusive. A detailed description of the components of sleep, as well as the underlying neurochemistry and circuitry is now slowly emerging.

Division of Sleep States

Sleep is generally considered to consist of two substates, ► **Rapid Eye Movement (REM)** and ► **non-Rapid Eye Movement (NREM)** sleep, which alternate to form a cycle lasting ~90 min in man (Fig. 1). ► **REM** and



Sleep. Figure 1 Slow wave sleep (SWS) and slow wave activity (SWA). The bottom panel illustrates a *hypnogram* showing the amount of time spent in the different sleep stages over the course of the night, using traditional visual scoring criteria. Each progression through the 5 stages (Stages 1–4 and REM) constitutes a sleep cycle. The top panel represents a fast Fourier transform of EEG data. The frequency band selected for power analysis was 0.75–4.5 Hz. This representation illustrates that SWA is present throughout the night, even when the sleeper does not reach stage 3 or 4 according to traditional visual scoring criteria (Dijk, unpublished data).

► **NREM** sleep can clearly be differentiated on the basis of a number of physiological variables including muscle tone, ► **electroencephalographic** (EEG) and electromyographic (EMG) features, and the presence or absence of rapid eye movements [1]. Distinct physiological roles for REM and NREM stages have been proposed, but compelling empirical data are scarce.

Real sleep in a living brain is a continuous state without clear transitions. Therefore, a temporal description of the waves and alterations in the amount of both frequencies and amplitudes should most likely be based on an analysis of these waveforms. However, for historical reasons, ► **sleep stages** are described as either REM or NREM stages 1–4 using visual scoring criteria based, in part, on the quantity and gross type of EEG waveforms per unit time. These are combined together graphically into a hypnogram (A graph detailing time along the x axis and *sleep stage* on the y axis. These are commonly used to depict the progression and relative proportion of the various *sleep stages* through a night's sleep) as shown in Fig. 1. NREM stages 1 and 2 have been described as light sleep, while stages 3 and 4 are often described as deep or ► **slow wave sleep (SWS)**. The hallmark waveform of SWS consists of rhythmic,

low-frequency waves (~ 0.5 –4.5 Hz). These electrical patterns also occur outside of stages 3 and 4, but to a much smaller extent, and are referred to as ► **slow wave activity (SWA)**. The amount of SWA in the ► **EEG** can be quantified by application of the Fourier transform to the complex signal, providing another objective means of evaluating the sleep process (Fig. 1).

SWS/SWA, in particular, may play an important role in somatic and cognitive restoration, including the consolidation of certain forms of procedural and declarative memory. A substantial diminution in the amount of SWS/SWA occurs across the human lifespan. This decline is beginning already in adolescence and middle-aged adults have only 25% of the SWS observed in young adults, whereas the elderly have almost none. While the clinical importance of these phenomena is unknown, it is reasonable to speculate that they may be related to the increase of sleep complaints associated with aging.

Regulation of Sleep

Sleep is thought to be under the control of two independent processes: one homeostatic (process S) and the other circadian (process C), which together

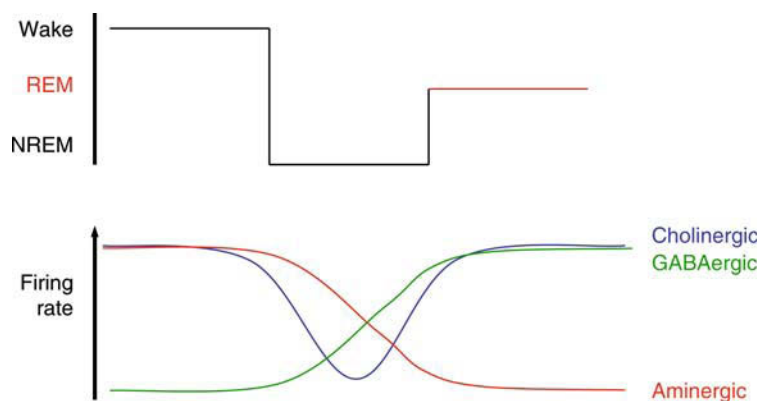
determine sleep propensity [2]. Process S represents a drive for sleep that increases progressively with wakefulness, and is most clearly seen under conditions of sleep deprivation, when “sleep debt” accumulates [2]. Failure to liquidate the debt through sleep results in profound neurobehavioral and cognitive deficits, as demonstrated during sleep restriction studies. Dissipation of the homeostatic drive for sleep is hypothesised to depend on time spent in NREM sleep. The amount of SWS/SWA in particular is thought to be an important marker of process S, since this parameter correlates strongly with sleep need.

Several gaps remain in our understanding of the neurobiological basis of the homeostatic process S. However, the data support a critical role for the ventrolateral preoptic area (VLPO), a dense cluster of neurones that inhibit ascending brainstem ►arousal pathways [3]. Specifically, the VLPO contains inhibitory (GABAergic and galanergic) efferent projections to arousal centres in the posterior hypothalamus and brain stem – including the tuberomammillary nucleus (TMN; histaminergic), locus coeruleus (noradrenergic) and the dorsal raphe (serotonergic). These ►arousal systems promote wakefulness via signals to thalamocortical and cortical pathways, with neuronal firing highest during wakefulness, declining through NREM sleep to quiescence during REM sleep (Fig. 2). In contrast, cholinergic activity is linked directly to the degree of cortical activation (observed in the EEG signal as high frequency, low amplitude activity), with the highest firing rates during waking and REM sleep [4]. While the mechanistic details are still under investigation, lesion studies have shown that VLPO neuronal activity (as indicated by expression of the

immediate-early gene *c-fos* and discharge rates) correlates closely with the onset, maintenance, and depth of NREM sleep. Conversely, during wake, VLPO activity is opposed by that of the major arousal systems. According to the model proposed by Saper et al., these reciprocal inhibitory projections produce a bistable system that resists change [3]. When pressure to change increases – during extended wakefulness, for example – the system flips from one state to another. The opposing, mutually inhibitory, actions of the ascending arousal and descending VLPO systems are stabilised by the orexin (hypocretin) system. During wakefulness, orexinergic activity is high, reinforcing the ascending arousal drive. In contrast, during sleep, this reinforcement is removed via VLPO inhibition of the orexinergic neurones [3].

Pathological conditions in which the VLPO system is weakened (i.e. less drive for sleep) could, therefore, result in more frequent changes between wakefulness and sleep, as has been demonstrated in animal models. Interestingly, elderly individuals have significantly reduced numbers – often by as much as 50% – of sleep-promoting VLPO neurones, an age-related loss of VLPO cells that may explain, at least partially, this population’s characteristic difficulty in falling and staying asleep.

Subserving process C is an endogenous ►circadian “clock” in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus that regulates the timing of daily transitions through sleep and wake. Incoming light from the retinal hypothalamic tract triggers the SCN to increase arousal levels. The arousal pathway is tightly regulated, as SCN innervation of the pineal gland also triggers the secretion of ►melatonin, which



Sleep. Figure 2 Relative activity of the aminergic (red), cholinergic (blue) and GABAergic systems (green) systems in relation to the arousal states. Cholinergic activity is directly correlated with cortical activation, as seen in the high frequency EEG activity recorded during the wake and REM states. The aminergic (NA, 5-HT, histamine) system is considered to be an arousal system, and accordingly activity is highest during wake and lowest during REM sleep. Aminergic activity is regulated by negative feedback from the GABAergic system emanating from the diffuse ventrolateral preoptic (VLPO) region. Figure constructed from information in the reviews by Jones [4] and Saper et al. [3].

substantially reduces this wake-promoting output. Melatonin is an important phase marker and regulator of the circadian clock, with secretion normally synchronised with nocturnal sleep and daytime wakefulness. However, melatonin is more correctly regarded as a “darkness hormone” rather than a sleep-inducing hormone, since it is secreted at night in nocturnal species as well as those active during daylight. The chronobiotic rather than hypnotic role of melatonin is further highlighted, however, by the fact that following ►phase shifts and in certain pathological conditions, melatonin secretion becomes uncoupled from sleep.

These insights into the structure of sleep and the neurobiological processes underlying it have yielded several targets for therapeutic intervention and provided novel methods by which to assess the differential effects of hypnotics on sleep-relevant neural circuits.

Sleep Disorders

Sleep disorders can be broadly divided into two categories: *dysomnias* and *parasomnias* (Table 1). *Dysomnias* are conditions of either too much sleep (hypersomnolence), or too little sleep (insomnia). ►Insomnias defined as difficulty in initiating or maintaining sleep, or poorer overall sleep quality. This is the most prevalent of sleep disorders, with as many as one in four reporting having experienced symptoms, and at least 10% of the population that consider this a chronic problem. ►Insomnia can be caused either by internal influences (e.g. dysfunction in the sleep–wake control mechanisms), external influences (e.g. changes in sleeping environment, light cycle, stimulant drugs) or as a secondary symptom of a comorbid disease (e.g. depression, anxiety, schizophrenia, Parkinson’s disease). ►Narcolepsy – a disease characterised by sudden, unpredictable transitions from wake to sleep and vice versa – is one of the few disorders for which a direct molecular basis has been established. Patients suffering from narcolepsy have been found to have under active orexin systems, usually as a result of cell loss, thereby removing the stabilising “finger on the switch” influence

of the system on arousal [3]. In contrast to the dysomnias, which are related to disruption of the sleep/wake control mechanisms, parasomnias are characterised by the presence of abnormal events during sleep. One intriguing parasomnia is that of REM sleep behaviour disorder, where patients experience an increased amount of REM sleep during which the normal inhibition of motor activity is absent. This manifests itself as sudden and violent outbursts during sleep (both vocal and physical) that can be immensely distressing for both the patient and those sleeping in the same environment. REM sleep behaviour disorder is often comorbid with Parkinson’s disease, and is currently thought to potentially represent an early indication of disease progression.

Pharmacological Intervention

Currently, the majority of all approved drugs for treating insomnia interact with the ►GABA_A receptor system. The major focus of the rest of this overview will therefore be on these types of drugs and the introduction of two novel approaches, either recently approved or in late stage development.

The most widely prescribed group of hypnotics all belong to the ►benzodiazepine receptor agonists (BzRAs). Biochemical and animal model studies have contributed greatly to our understanding of the receptor interactions by which the BzRAs achieve their sedative–►hypnotic effects. However, a clear understanding of exactly how these mechanisms contribute to the transition between different brain states is still rudimentary. At the receptor level, BzRAs allosterically enhance the ability of GABA to increase inhibitory chloride currents through the ion channel intrinsic to GABA_A receptors [5]. Diazepam, temazepam and other classical benzodiazepines bind at the interface between γ_2 and α subunits, within the same binding pocket as zolpidem and other non-benzodiazepine hypnotics, so called because of their chemically distinct structure. Classical benzodiazepines demonstrate roughly equivalent binding affinities for all γ_2 -containing GABA_A receptor subtypes, while the non-benzodiazepines zolpidem and zaleplon demonstrate about tenfold binding selectivity for the $\alpha_1\beta\gamma$ subtype. The extent to which this ►binding selectivity translates into ►functional selectivity in vivo is a matter of intense interest. Indeed, using a variety of expression systems, investigators have measured the enhancement of GABA-evoked chloride conductance to characterise the potency and efficacy of these types of compound. Results suggest that in the context of physiological concentrations, the majority of BzRAs demonstrate little in the way of subtype selectivity.

BzRAs significantly reduce ►sleep latency and the number and duration of awakenings, resulting in increased total sleep and improved sleep continuity.

Sleep. Table 1 Examples of various dysomnias and parasomnias

Dysomnias	Parasomnias
►Insomnia	Sleep walking
►Narcolepsy	Bedwetting
►Restless legs syndrome	Sleep talking
►Sleep apnea	►REM (sleep) behaviour disorder
►Periodic limb movements	
Circadian sleep disorders	

Their clinical profile, however, is marked by residual daytime sleepiness, ►**anterograde amnesia**, and, importantly, withdrawal symptoms on discontinuation of therapy, reflecting their significant potential for physical dependence and abuse. Studies in baboons also demonstrate reinforcing properties underlying their abuse liability. The relatively non-selective binding and long half-lives of the benzodiazepines – ranging from 47–100 h for flurazepam to 2–5 h for triazolam and 8–20 h for temazepam, the two short-acting benzodiazepines currently indicated for insomnia – are believed to account for their significant residual effects.

The non-benzodiazepine BzRA hypnotics have pharmacological profiles distinct from those of the classical benzodiazepines, and are regarded as an important advance in the treatment of insomnia. Firm generalisations about clinically important differences between these and classical benzodiazepines cannot be made, however, given the paucity of head-to-head clinical comparisons at equipotent doses. Pharmacokinetic properties such as bioavailability, plasma half-life, elimination rate, and blood–brain barrier penetration more transparently influence the onset and duration of hypnotic effects and, to some extent, next-day neuro-behavioral and cognitive consequences. Compounds in this class include zolpidem, zopiclone, eszopiclone, indiplon and zaleplon. The receptor subtype selectivity and improved pharmacokinetic profile of the non-benzodiazepines compared with the benzodiazepines have yielded some clinically important benefits. Nonetheless, the abuse liability of these compounds, particularly that of zaleplon, zopiclone and eszopiclone, has been suggested to be similar to that of benzodiazepines like temazepam and triazolam. In addition, at least at higher doses, tolerability concerns exist even with the newer medications, particularly with respect to residual and amnestic effects. There is thus interest in developing compounds that target different receptors, having more specific effects on the regulation of sleep without these side effects.

Several novel approaches to the treatment of insomnia have attracted much attention among researchers.

Gaboxadol is a Selective ►**Extrasynaptic GABA_A receptor** Agonist (SEGA), a new class of GABAergic drug that activates receptors located primarily at regions outside of the synapse. In functional assays, gaboxadol selectively elicits chloride conductance through δ -subunit containing ►**GABA_A receptor** subtypes. The biophysical basis of this functional selectivity remains an active area of research. Notably, the BzRAs are unable to bind δ -containing GABA_A receptor subtypes, suggesting a completely different target to that of BzRAs. These ►**extrasynaptic** receptors show a limited CNS distribution and are particularly enriched in the cerebral cortex, limbic system and thalamus. Recent studies have demonstrated that gaboxadol enhances a

bicuculline-sensitive but zolpidem- and midazolam-insensitive tonic current in thalamic relay neurones in mice. Whether this extrasynaptic conductance is linked to the ►**thalamocortical synchronisation** that drives SWS and SWA is a question of particular interest, since gaboxadol significantly increases SWS and SWA in animals and humans. In drug discrimination studies, rats and primates trained to recognise BzRAs did not respond when administered gaboxadol, demonstrating a qualitative difference in the effects of gaboxadol compared with the BzRAs, and highlighting the novelty of this compound with respect to the older medications.

Ramelteon, a selective melatonin receptor agonist with high affinity for MT₁ and MT₂ receptors located in the SCN, has recently received FDA approval for patients with difficulty falling asleep. Studies in animals indicate that ramelteon significantly decreases wakefulness and increases SWS and ►**REM** compared with placebo. Beneficial effects of ramelteon on sleep have been confirmed in clinical studies. However, in contrast to findings in animals, clinical studies have not indicated an increase in SWS with ramelteon. Neither ►**rebound insomnia** nor withdrawal effects have been observed following chronic administration. Ramelteon thus lacks abuse potential, a direct consequence of its distinct mode of action, and is therefore not a scheduled drug.

Serotonin_{2A} receptors have also been the focus of sleep enhancing agents on the basis that they promote SWS in both animal models as well as clinical trials, without the side effects associated with BzRAs. The precise interactions of these compounds within the sleep control pathway remain to be elucidated.

In addition to insomnia, narcolepsy is also relatively well treated by the range of medications available today. Daytime sleep intrusions are generally combated using stimulants such as amphetamine or modafinil, and cataplexy (sudden loss of muscle tone during the sleep episode) using some of the older antidepressant compounds. A more novel approach is offered by the hypnotic sodium oxybate, which is reported to alleviate symptoms of narcolepsy by improving the continuity of the night time sleep. The wake promoting medication modafinil is of particular note, in that it appears to be able to elicit periods of extended wakefulness, without causing rebound hypersomnolence.

Much has been discovered recently regarding the molecular mechanisms and pathways involved in sleep and its associated disorders. It is hoped that this knowledge will lead to improved therapeutics lacking the side effects of currently available drugs.

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Sleep Apnea

A condition which is characterised by pauses in breathing during sleep. Each pause lasts long enough to miss several breaths and is normally repeated throughout the night. Can be caused centrally by a disruption in respiratory control or obstruction of airways during sleep through lack of muscular tone or some other obstruction. Sleep apnea can cause deprivation of deep sleep and daytime sleepiness.

► Sleep

Sleep Continuity

A measure used to assess sleep quality, usually determined by means of *polysomnography*. A parameter such as “wake after sleep onset” (WASO) is often used to describe sleep continuity.

► Sleep

Sleep (Onset) Latency

Time taken to fall asleep from full wakefulness. Used clinically as a measure of insomnia, and to determine the effectiveness of *hypnotic* medications.

► Sleep

Sleep Quality

Determinations of the quality of sleep can either be made based on patient report (i.e. perceived sleep quality), using standardised questionnaires, or by more

objective measurements (e.g. *sleep latency*, *sleep continuity*) obtained via *polysomnographic* recording of the sleep period.

► Sleep

Sleep Stages

Since the publication of the *Standardised Sleep Manual* by Rechtschaffen and Kales (Eds) in 1968, human sleep has been described using a classification system based on a combination of EEG, EMG and EOG features. The acquisition and use of such data is known as *polysomnography*. Note, that when including the state of WAKE in the classification, these are most accurately described as *arousal states* in place of sleep stages.

Electroencephalographic (EEG), electro-oculographic (EOG) and electromyographic (EMG) feature characteristic of the stages of sleep (NREM stages 1–4 and REM) and wake. The distinctions between REM and NREM sleep are dramatic and qualitative, including differences in EEG and EOG patterns, changes in heart

Stage	EEG characteristics	EOG	EMG muscle activity
W	Predominant alpha activity (more than 50% of the epoch) mixed with EEG beta	Slow and rapid	High
1	Alpha activity is replaced by predominant low-voltage, mixed-frequency background activity sometimes with vertex sharp waves	Slow	Decreased from wake
2	Sleep spindles and K complexes in a background EEG that has less than 20% delta activity	None	Decreased from wake
3	Slow waves (EEG delta activity) comprise 20%–50% of the epoch; sleep spindles usually are present	None	Decreased from wake
4	More than 50% of the epoch has EEG delta activity	None	Decreased from wake

rate and respiration, and the presence or absence of muscle tone. Distinctions between NREM stages are more arbitrary and are centred on a progressive increase in amplitude and slowing of the EEG with successive stages. “Depth” of sleep, as indexed by an increase in the arousal threshold, increases with progression through NREM stages.

► Sleep

Sleeping Sickness (African)

Vector-born infectious disease caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*.

► Antiprotozoal Drugs

Slow Wave Activity

Synonyms

SWA

Definition

Oscillations in the EEG of between 0.5 and 4 Hz, sometimes also called delta activity. SWA is a hallmark of the sleeping brain, and is most prevalent in the deepest *sleep stages* (stages 3–4). The slow oscillations arise from widespread synchrony of neuronal firing, particularly in the thalamocortical circuits.

► Sleep

Slow Wave Sleep

Generic term usually applied to the deeper *stages* of NREM sleep (stages 3 and 4), so called because of the high proportion of slow wave activity (SWA).

► Sleep

Smad

(Mothers against decapentaplegic homolog) A group of related intracellular proteins with a key role in the transforming growth factor- β signaling cascade composed of three domains: an N-terminal Mad homology 1 (MH1) domain, a nonconserved linker region, as well as a C-terminal MH2 domain. There are at least eight different Smad proteins in vertebrates responsible for transmitting TGF- β signals from the cell surface to the nucleus and are grouped into three classes: (i) Receptor-activated Smads (R-Smads) – Smad1, Smad2, Smad3, Smad5 and Smad8; (ii) Comediator Smad – Smad4; and (iii) Inhibitory Smads (I-Smads) – Smad6 and Smad7.

► Transforming Growth Factor-Beta

Small G Proteins

► Small GTPases

Small GTPases

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Synonyms

Low molecular mass GTPases; Small G proteins

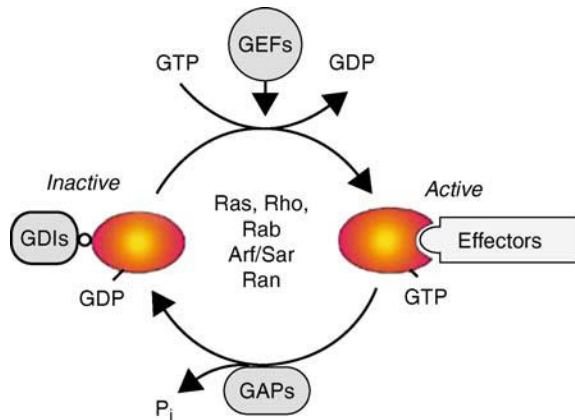
Definition

Small GTPases are monomeric 20 to 40 kD GTP-binding proteins that interconvert between an active (GTP-bound) and an inactive (GDP-bound) state. As molecular switches they are involved in the regulation of complex cellular processes.

Basic Characteristics

Regulation

Activation of small GTPases occurs by GDP/GTP exchange catalyzed by ► [guanine nucleotide exchange factors \(GEFs\)](#) (Fig. 1). They stimulate the dissociation of GDP in response to an upstream signal and results in the binding of GTP. In the GTP-bound form the GTPases are active, and bind to and activate a number of effector molecules. The small G proteins are able to



Small GTPases. Figure 1 The GTPase cycle: GTPases are inactive in the GDP-bound form. In a complex with guanine nucleotide dissociation inhibitors (GDIs) the inactive form is stabilized (note, not all small GTPases are regulated by GDIs, e.g. not Ras, Ran and Arf subfamily proteins). Guanine nucleotide exchange factors (GEFs) cause the release of GDP and binding of GTP, and thereby the activation of the proteins. The active state of the GTPases is turned off by GTP hydrolysis catalyzed by GTPase activating proteins (GAPs).

hydrolyse the bound nucleotide to GDP. This inactivation step is accelerated by **GTPase activating proteins (GAPs)**. In the GDP-bound form the GTPases are inactive and some bind then to **guanine nucleotide dissociation inhibitors (GDIs)** that stabilize the inactive form and cover the lipid modification of the GTPase as a cytosolic complex. Novel roles of GDI in GTPase regulation like the delivery of GTPases to specific sites within the cell are discussed. Moreover, local synthesis and degradation of GTPases seem to play additional roles in the spatial regulation of small GTPases. In mammalian cells, each family of GTPase regulating proteins comprise numerous members which are more or less specific for individual GTPases, cell types, GTPase functions and signal pathways.

General Structural Properties

All small GTPases are folded in a similar way. They possess four consensus amino acid sequences in common, which are involved in nucleotide binding and hydrolysis: GXXXXGK, DXXG, NKXD and EXSAX. Two highly flexible regions (Switch I and Switch II regions) determine the nucleotide-dependent activity state of the GTPases and the protein–protein interactions with effectors and regulatory proteins.

Post-Translational Modification

All small GTPases (except Ran) are post-translationally modified. Most important is the isoprenylation of the C-terminus. The type of modification is determined by

the COOH-terminal amino acid sequence. GTPases with a C-terminal CAAX-box (A = aliphatic amino acid, X = any amino acid) are farnesylated at the cysteine residue followed by the proteolytic degradation of the last three amino acids and subsequent methylation of the carboxy-terminus. In the case of a CAAL or CAC, the cysteines are modified by geranylgeranylation. In some cases an additional cysteine is palmitoylated or N-terminal myristoylation occurs. All these post-translational modifications allow the interaction of GTPases with the phospholipid bilayer. Lipid modification of the GTPases is required for their membrane localization and GDI binding.

Families

The superfamily of small GTPases consists of more than 100 members from yeast to human with more than 80 members expressed in mammalian cells. Based on structural and functional similarities the GTPases are subdivided into five major classes.

Ras GTPases

The mammalian family of Ras GTPases consists of more than 15 members which share high homology to each other and include Ha-Ras, Ki-Ras, N-Ras, R-Ras, Rap, Ral, Rheb, Rin and Rit proteins (1). Ras proteins have achieved attention with the discovery that they contain point mutations in 15% of all human tumours (more than 90% in pancreatic tumours), leading to the exchange of conserved amino acids, e.g. at positions 12 and 61. Amino acid exchanges at these positions block the GTP hydrolase activity of the GTPases, resulting in constitutive activation. Ras GTPases are involved in signal transduction of proliferation and/or differentiation. They couple receptor tyrosine kinases with a protein kinase cascade termed Raf/ERK kinase pathway (also known as MAP kinase cascade). Activation of this pathway leads to phosphorylation and activation of transcription factors like Elk-1, and stimulate gene expression. Activated Ras has been shown to transform culture cells and to produce tumours in nude mice. Besides the Raf kinase, the RalGDS, which is an activator of the Ral subfamily proteins, and the PI3 kinase, which is involved in inositol signalling, are important effectors of Ras. Ral GTPases (<50% identical with Ras) control cell proliferation, Ras-mediated cell transformation, vesicle traffic, phospholipase D and cytoskeleton organization. Rap GTPases have been identified in a screen for cDNAs that are able to revert the transforming phenotype of Ki-Ras (Kirsten Ras) and, therefore, were also termed K-rev proteins.

Rho GTPases

Members (>20) of the Rho family of GTPases, including RhoA, B and C, Cdc42 and Rac1, 2, 3,

share more than 50% sequence identity. The GTPases are important regulators of the actin cytoskeleton (2). RhoA regulates the formation of actin stress fibres, whereas Cdc42 is known to induce filopodia. Rac is involved in the formation of lamellipodia and membrane ruffles. Rho GTPases are involved in migration, phagocytosis, endo- and exocytosis, cell-cell and cell-matrix contact. Rac regulates NADPH oxidase. Furthermore, Rho GTPases are involved in transcriptional activation, cell transformation and apoptosis. Rho GTPases are regulated by a large number of GEFs and GAPs (>60 members of each family have been identified), suggesting function-specific regulation of the activity state.

A subfamily of Rho proteins, the Rnd family of small GTPases, are always GTP-bound and seem to be regulated by expression and localization rather than by nucleotide exchange and hydrolysis. Many Rho GTPase effectors have been identified, including protein and lipid kinases, phospholipase D and numerous adaptor proteins. One of the best characterized effector of RhoA is Rho kinase, which phosphorylates and inactivates myosin phosphatase; thereby RhoA causes activation of actomyosin complexes. Rho proteins are preferred targets of bacterial protein toxins (►bacterial toxins).

Rab GTPases

The largest family of small GTPases with more than 40 members identified are the Rab GTPases (3). Rab proteins are important regulators of specific steps of vesicle trafficking, including budding, targeting, docking and fusion with acceptor membranes. Each Rab protein has an organelle-specific subcellular localization and seems to be functionally specialized. Rab1A and Rab1B are two of the most extensively studied members of the Rab family. Both proteins are found in membranes of the ER, Golgi apparatus and intermediate vesicles between these compartments. They appear to function in the anterograde trafficking of proteins from the ER to the Golgi compartment. Rab4 and Rab5 are present on early endosomes and are involved in the endocytic process, whereas Rab6 is localized at the Golgi apparatus regulating processes of the secretory pathway. One of the best studied members of the Rab protein family is Rab3a. This GTPase is a key regulator of Ca^{2+} -induced exocytosis, particularly in nerve terminals. Several effectors of Rab proteins like Rabphilin, Rabaptin and Rim have been identified and characterized as essential for vesicle trafficking. Recently, the Rab effector Rabkinesin6 has been identified that links Rab proteins to the microtubule cytoskeleton. Rabkinesin6 may be the motor driving vesicles along microtubules from the Golgi apparatus to the periphery.

Arf/Sar1 GTPases

The name Arf (ADP-ribosylation factor) stems from its discovery as a cytosolic factor with the ability to enhance the ADP-ribosylation of the α -subunit of the G protein G_s by ►cholera toxin. Arf is known to regulate phospholipid metabolism. Studies with dominant active or dominant negative mutants of Arf proteins in mammalian cells suggest the involvement of these GTPases in the trafficking of coated vesicles, and it is now known that Arf1 regulates the formation of COPI-coated vesicles for retrograde transport between Golgi apparatus and endoplasmic reticulum (4). Sar1, which is 37% identical to Arf1, is needed for the assembly of COPII proteins for vesicle transport in the opposite direction. Taken together, Arf and Sar proteins play crucial roles in the recruitment of COP components to vesicles thereby regulating vesicle budding. In contrast to the other small GTPases, Arf/Sar1 proteins are not regulated by GDI proteins, whereas different GEF and GAP proteins have been identified. Myristoylation of Arf proteins at the N-terminus is required for its membrane localization.

Ran GTPases

In mammalian cells there is only one *Ran* gene, which was discovered as a Ras-like gene (Ran: Ras-related nuclear protein) (5). In contrast, in yeast more than one related *Ran* genes have been identified. The predominant nuclear localization of the GTPase was the first hint that Ran is involved in nucleocytoplasmic transport processes. Interestingly, the only Ran GEF present in mammalian cells, RCC1 (regulator of chromatin condensation), is localized exclusively in the nucleus, whereas the single Ran GAP (Ran GAP1) is in the cytoplasm. This specialized localization of the regulators is the prerequisite for the asymmetric distribution of the GDP- and GTP-bound form of Ran and for its role as a nucleocytoplasmic transporter. In contrast to other GTPases, the activity of Ran is dependent on the gradient of the GTP-bound GTPase from cytoplasm to nucleoplasm that allows the transport of cargo proteins. Ran is involved in nuclear import as well as in export of proteins through the ►nuclear pore complex. Both processes require the formation of protein complexes, including Ran, the cargo protein and Ran-binding proteins like ►importins or ►exportins. In addition to its transporter function, Ran has been shown to participate in microtubule organization during the M phase of the cell cycle.

Cascades and Cross-Talk

Small GTPases are not isolated molecular switches regulating cellular processes. Signalling cascades within one subfamily as well as cross-talk between

members of different subfamilies are known. For example, Cdc42/Rac/Rho are sequentially activated after extracellular stimuli in quiescent Swiss 3T3 cells. Moreover, reciprocal modulation between Rho GTPases has been described. Ras and Rho proteins act in a cooperative manner in Ras-induced transforming. A further example of cross-talk between GTPase families is the cooperative function of Rho and Rab proteins during cell migration, with Rho proteins controlling the actin cytoskeleton, and Rab proteins regulating vesicular traffic for the recruitment of membrane material, and the recycling of proteins like integrins. Arfaptn connects signalling via Arf and Rac in regulating fundamental processes like endocytosis and secretion.

Drugs

Small GTPases, among other activities, regulate cell growth, neurite outgrowth and signalling of immune cells involved in inflammation. Pharmacological modulation of the activity of small GTPases is thus a useful aim in cancer and anti-inflammatory therapies. Farnesyltransferase inhibitors can be used to block the post-translational modification of the GTPases, which for example is essential for the transforming activity of Ras or Rho GTPases. Moreover, inhibitors of GTPase effectors like Rho kinase inhibitors have been generated. Such agents are at present in clinical trials.

- Bacterial Toxins
- Exocytosis
- Intracellular Transport
- Growth Factors

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Smooth Muscle Tone Regulation

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Synonyms

Regulation of smooth muscle contractility

Definition

Following organs contain as major functional part smooth muscles layers: Arterial and venous vessels; lung and bronchia; oesophagus, stomach, small and large intestine; urinary tract and bladder; uterus. Hormones, locally released transmitters and shear stress or pressure regulate the tonus of these organs. Each organ has a slightly different regulation of its contractility, but the basis for this regulation, i.e. the intracellular signalling pathways, are very similar or identical. This article will focus on major findings that may be identical in all smooth muscles.

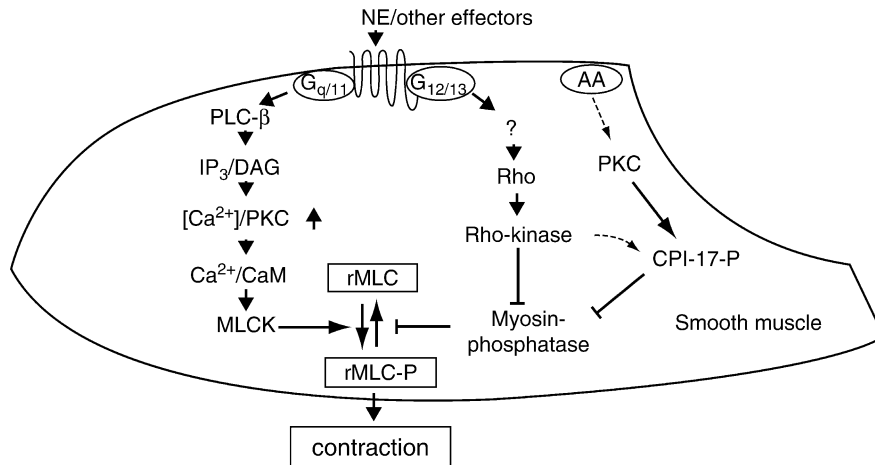
Basic Mechanisms

Key mechanism of smooth muscle tone regulation is the phosphorylation of Ser-19 of the ►regulatory myosin light chain II (rMLC) [1]. Phosphorylation and dephosphorylation is catalysed by ►myosin light chain kinase (MLCK) and the type 1 ►myosin phosphatase (MLCP), respectively. Calcium-dependent and calcium-independent signal pathways regulate the activity of both enzymes and thereby the phosphorylation status of rMLC. An increase in the cytosolic calcium concentration leads to phosphorylation of the rMLC and contraction within 4 s. The correlation between percent phosphorylated rMLC and developed force is quite variable. Maximal force can be attained at 0.2–0.3 mol phosphate per mol rMLC. Phosphorylation can decline during maintenance of tension suggesting that even dephosphorylated cross-bridges can contribute to force maintenance.

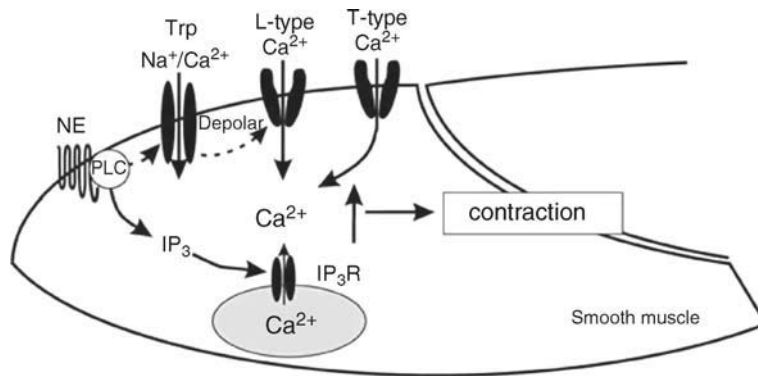
Calcium-dependent regulation involves the calcium-calmodulin complex that activates smooth muscle MLCK, a monomer of approximately 135 kDa. Dephosphorylation is initiated by MLCP. MLCP is a complex of three proteins: a 110–130 kDa myosin phosphatase targeting and regulatory subunit (MYPT1), a 37 kDa catalytic subunit (PP-1C) and a 20 kDa subunit of unknown function. In most cases, calcium-independent regulation of smooth muscle tone is achieved by inhibition of MLCP activity at constant calcium level inducing an increase in phospho-rMLC and contraction (Fig. 1).

Calcium-Dependent Contraction

Different agonists such as norepinephrine, acetylcholine or angiotensin II activate smooth muscle contraction by binding to a heptahelical receptor, i.e. α -adrenergic, muscarinic or AT-1 receptors, followed by an increase in cytosolic calcium (Fig. 1 and 2). The activation of the trimeric G Proteins G_q or G_{11} increases the activity of phospholipase C β (PLC) generating inositol trisphosphate (IP $_3$) and diacylglycerol (DAG) and other fatty acid derived compounds. Classical findings suggested that IP $_3$ stimulates calcium release from intracellular stores that binds to calmodulin and activates MLCK. This simple scheme is not in line with the fact that a block of the ►L-type Ca $^{2+}$ channel inhibits



Smooth Muscle Tone Regulation. Figure 1 Mechanisms leading to agonist stimulated calcium-dependent and calcium-independent contraction of smooth muscle. NE, norepinephrine. See text for the other abbreviations.



Smooth Muscle Tone Regulation. Figure 2 Membrane mechanisms leading to an increase in cytosolic calcium concentration. depolar, depolarisation of the membrane; see text for abbreviations.

contraction. The importance of the L-type calcium channel is further supported by genetic deletion of the corresponding $Ca_v1.2$ gene. Mice lacking the smooth muscle $Ca_v1.2$ channel have severe difficulties to contract intestinal and other smooth muscle. It is therefore likely that activation of a heptahelical receptor leads to depolarisation of the membrane and activation of the L-type calcium channels (Fig. 2). Possible candidates are **TRP channels** (most likely TRPC6) activated either by DAG or by interaction with empty IP_3 -stores. The inflowing cations may depolarise the membrane to potentials that activate T- and thereafter L-type calcium channels or directly L-type channels. A second channel depolarising the membrane is the calcium-activated chloride channel present in many smooth muscle cells. Calcium released from IP_3 -stores or flowing in through TRP channels could activate this chloride channel and depolarise thereby the membrane. The L-type calcium channel provides calcium to trigger calcium release from ryanodine receptor controlled

calcium stores and for refilling various intracellular calcium stores ([2]). The mechanism behind pressure or shear stress induced contraction is unsolved, but may again involve TRP channels.

Calcium-Independent Contraction

Agonist-activated receptors can induce contraction at a constant intracellular calcium concentration [3], if the receptor activates the G proteins G_{12} or G_{13} (Fig. 1). Activation of these G proteins recruits the monomeric GTPase **Rho** to the membrane, where Rho exchanges GDP against GTP and activates **Rho-kinase**. By a still unsolved cascade eventually involving ZIP kinase, the MYPT1 subunit of MLCP is phosphorylated at Thr-697 and Thr-854 (rat MYPT1) which reaction inhibits MLCP activity. Since the activity of MLCK is not affected by this cascade, rMLC is phosphorylated to a higher level. Phosphorylation and inhibition of MLCP activity is only observed, if a central exon of MYPT1 is present. These results could explain the old finding that certain agonists

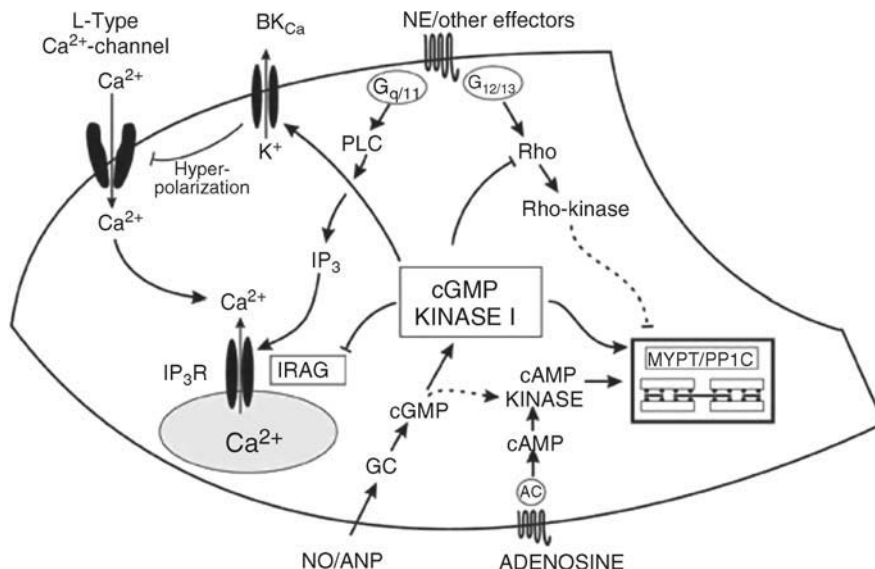
induce calcium sensitization of the contractile machinery in most but not all smooth muscles. MLCP activity is also affected by a smooth muscle specific inhibitor protein of PP-1C, named **CPI-17**. Protein kinase C (PKC) phosphorylates CPI-17, which becomes then a high affinity inhibitor of the catalytic subunit of MLCP. The nature of the PKC subtype is not clear. It is possible that it is one of the atypical PKC enzymes that is activated directly (?) by arachidonic acid (AA). Rho-kinase which phosphorylates CPI-17 in vitro apparently may affect directly in vivo the phosphorylation status of CPI-17 [4]. Arachidonic acid inhibits dephosphorylation of MLCs, i. e. by a second mechanism by dissociating the MLCP holoenzyme.

Relaxation of Smooth Muscle

The major relaxing transmitters are those that elevate the cAMP or cGMP concentration (Fig. 3). Adenosine stimulates the activity of cAMP kinase. The next step is not clear, but evidence has been accumulated that cAMP kinase decreases the calcium sensitivity of the contractile machinery. In vitro, cAMP kinase phosphorylated MLCK and decreased thereby the affinity of MLCK for calcium-calmodulin. However, this regulation does not occur in intact smooth muscle. Possible other substrate candidates for cAMP kinase are the heat stable protein HSP 20, (A heat stable protein of 20 kDa that is phosphorylated by cGMP kinase. It has been postulated that phospho-HSP 20 interferes with the interaction between actin and myosin allowing thereby smooth muscle relaxation without dephosphorylation of the rMLC.) Rho A and MLCP that are phosphorylated also by **cGMP kinase I** (Fig. 3).

A major relaxing factor is NO, a signal molecule synthesized by three different **NO synthases (NOS)**. NO synthesized in the endothelial layer of the vessels diffuses into the smooth muscle layer, where NO activates soluble guanylate cyclase (GC) and generates high concentrations of cGMP. In non-vascular systems such as the intestinal smooth muscle, NO is released from non-adrenergic, noncholinergic neurons. An alternative pathway for the production of cGMP, is the stimulation of particulate GC by the **natriuretic peptides ANF and BNF**. ANF and BNF are released from cardiac atrial and ventricular muscle, respectively, and lower blood pressure. The effects of the natriuretic peptides are mediated through cGMP and cGMP kinase I, whereas NO has effects which are not mediated by cGMP kinase I.

Smooth muscle contains the two cGMP kinase isozymes Ia and Ib and a number of identified substrates [5]. The NO/cGMP/cGMP kinase pathway interferes with the calcium-dependent and the calcium-independent contraction. A number of researchers have shown that cGMP-dependent phosphorylation of the **BK_{Ca} channel** increases its open probability resulting in hyperpolarization of the membrane potential and closure of voltage-dependent calcium channels. The activity of the BK_{Ca} channel is upregulated by the intracellular calcium concentration establishing a negative feedback loop. It is well established that cGMP kinase decreases the release of calcium from intracellular stores. Recently, it was found that cGMP kinase Ib is associated with the IP₃ receptor type 1 and the 130 kDa protein **IRAG**. Phosphorylation of IRAG inhibited the release of calcium from IP₃-sensitive stores in COS cells. However, isozyme-specific reconstitution of cGMP kinase I



Smooth Muscle Tone Regulation. Figure 3 Major mechanisms leading to relaxation of smooth muscle. See text for the abbreviations.

deficient mice suggested that, in murine aortic smooth muscle cells, cGMP kinase I α and I β lowered norepinephrine-stimulated increases in the cytosolic calcium concentrations. This result is in line with the recent notion, which calcium-dependent contraction of smooth muscle requires membrane depolarisation and calcium influx through membrane localized ion channels (see above). It is possible that the IP₃-sensitive calcium pool associated with IRAG and cGKI β controls other smooth muscle functions such as phenotype changes and smooth muscle growth.

Recently it was demonstrated that cGMP kinase I inhibits also smooth muscle contraction due to the calcium-insensitive pathway. A possible mechanism could be phosphorylation of Rho by cGMP kinase I. The phosphorylation site is identical with a cAMP kinase site identified in Rho from non-smooth muscle cells. It was reported that phosphorylation of Rho by cGMP kinase I prevents membrane association of Rho, that is required to stimulate the GDP/GTP exchange. Alternatively, phosphorylation of telokin may interfere with the calcium sensitisation of contraction. cGMP kinase I α interacts specifically with a leucine zipper present at the C-terminus of MYPT1. Depending on the tissue, this leucine zipper is present or not. MLCP activity increased when MYPT1 is phosphorylated by cGMP kinase I α at Ser-696.

Pharmacological Intervention

A large number of drugs interfere with the smooth muscle contraction. These compounds lower blood pressure and are referred to as antihypertensive. In this section, only those compounds will be mentioned that have a direct effect on smooth muscle tone. Phenylephrine is an agonist on most smooth muscles and activates α_1 adrenoceptors. Carbachol is an agonist on some smooth muscles and activates contraction through muscarinic receptors. Blockers of the α_1 -adrenoceptors such as prazosin and urapidil are competitive inhibitors of the α_1 -receptor in vascular and bladder smooth muscle. Phenoxybenzamine is an irreversible blocker of α_1 receptors and phentolamine blocks α_1 and α_2 receptors. ►**Ca²⁺ channel blockers** such as the dihydropyridines, phenylalkylamines and benzothiazepines lower smooth muscle tone by blocking the L-type calcium channel.

►**Nitrates** (glycerol trinitrate, isosorbit dinitrate, pentaerythritol tetranitrate, molsidomine, sodium nitropruside), that generate NO, increase cGMP concentrations and lower smooth muscle tone by activation of cGMP kinase I. Nitrates relax in vivo mainly the capacitative part of the circulation system, i.e. the venous part. ►**Sildenafil**, a specific inhibitor of phosphodiesterase V, a cGMP hydrolysing enzyme, increases cGMP in the corpus cavernosum and lowers together with nitrates the blood pressure by the combined effect on cGMP level. An additional potentially important drug family are

compounds that stimulate the soluble guanylyl cyclase independent of NO [6] and lower elevated blood pressure. The inhibitor of Rho-kinase, ►**Y-27632**, lowers elevated blood pressure in hypertensive animals, without affecting significantly the blood pressure of non-hypertensive animals ([7]).

►Nitric Oxide

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SMURF

Synonyms

Smad ubiquitin regulatory factor

Definition

Smad specific E3 ubiquitin ligases that associate with and lead to the degradation of the cytoplasmic receptor activated R-Smads. This regulatory protein serves to keep the available R-Smad pools low. Smurfs have been shown to regulate PRAJA ubiquitination of ELF which can be displaced from the TGF- β signaling pathway. Additionally, Smurfs can ubiquitinate phosphorylated nuclear R-Smads leading to their degradation. Through the nuclear R-Smad/Smurf complex ubiquitination of transcriptional repressors, the repressors can be down-regulated allowing the removal of inhibitory activities from target genes that require transcriptional induction by Smad complexes.

►Transforming Growth Factor-Beta

SNAPs

SNAPs is an acronym for soluble NSF attachment proteins. They were originally discovered as cofactors for NSF that mediate the membrane binding of NSF in *in vitro* transport assays. Several isoforms of SNAPs exist in mammalian cells. SNAPs are also highly conserved proteins. Crystallographic studies indicated that the proteins form a very stiff and twisted sheet that is formed by a series of antiparallel and tightly packed helices connected by short loops.

► Exocytosis

SNARE Proteins

SNAREs is an acronym for soluble NSF acceptor protein receptors. They are a superfamily of small and mostly membrane-bound proteins that are distinguished by the presence of a conserved stretch of 60 amino acids referred to as a SNARE motif. With few exceptions, a single transmembrane domain is located adjacent to the SNARE motif at the C-terminal end. Many SNAREs possess in addition an independently folded N-terminal domain whose structures are more diverse.

SNARE motifs spontaneously assemble into SNARE complexes. These consist of a bundle of four intertwined α -helices that are connected by a total of 16 layers of mostly hydrophobic amino acid side chains. In the middle of the bundle, there is a highly conserved and polar “0-layer” consisting of three glutamine and one arginine residue. These residues are among the most conserved in the SNARE superfamily and led to a classification of SNAREs into Q- and R-SNAREs, respectively. Different fusion steps require different sets of SNAREs but some SNAREs can participate in different complexes, and some fusion steps involve several SNARE complexes that appear to operate in parallel and independently.

In vitro, SNARE-complex formation is irreversible. Disassembly requires the concerted action of the chaperone-like ATPase NSF and SNAPs.

► Exocytosis
► Synaptic Transmission

SNF1 Complex Au2 (Fungi)

► AMP-Activated Protein Kinase

SNPs

► Single Nucleotide Polymorphisms

SNRIs

► Selective Noradrenaline Reuptake Inhibitors

SOCS

Suppressors of cytokine signaling are a family of cytokine-inducible proteins that inhibit JAK kinases.

► The JAK-STAT-Pathway

SOD

► Superoxide Dismutase

Solid Phase Synthesis

Solid phase synthesis is a polymer-supported or solid-supported synthesis, i.e., stepwise construction of product molecules attached to an insoluble organic or inorganic polymer.

► Combinatorial Chemistry

Soluble Guanylyl Cyclase

The enzyme guanylyl cyclase produces the second messenger guanosine monophosphate (3', 5'-cyclic GMP, cGMP) from guanosine triphosphate (GTP).

The soluble isoform is the primary target of the signaling molecule NO.

►Guanylyl Cyclase

Solute Carrier

►Table appendix: Membrane Transport Proteins

Somatomedins

Somatomedins are polypeptide mediators produced in response to growth hormone in the liver, e.g. insulin-like growth factors (IGFs). In particular, IGF-1 is the main mediator of growth hormone action.

Somatostatin

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Synonyms

Somatostatin; SOM; SST14; SST28; Somatotropin release inhibitory factor (SRIF); Growth hormone release-inhibiting factor

Definition

Somatostatin is a regulatory cyclic peptide, which has originally been described as a hypothalamic growth hormone release-inhibiting factor. It is produced throughout the central nervous system (CNS) as well as in secretory cells of the periphery and mediates its regulatory functions on cellular processes such as neurotransmission, smooth muscle contraction, secretion and cell proliferation via a family of seven transmembrane domain G-protein-coupled receptors termed sst₁₋₅.

Basic Characteristics

Biosynthesis

The human somatostatin gene is located on chromosome 3q28 and contains a single intron of 876 bp in its coding sequence. Its 5' upstream region includes several

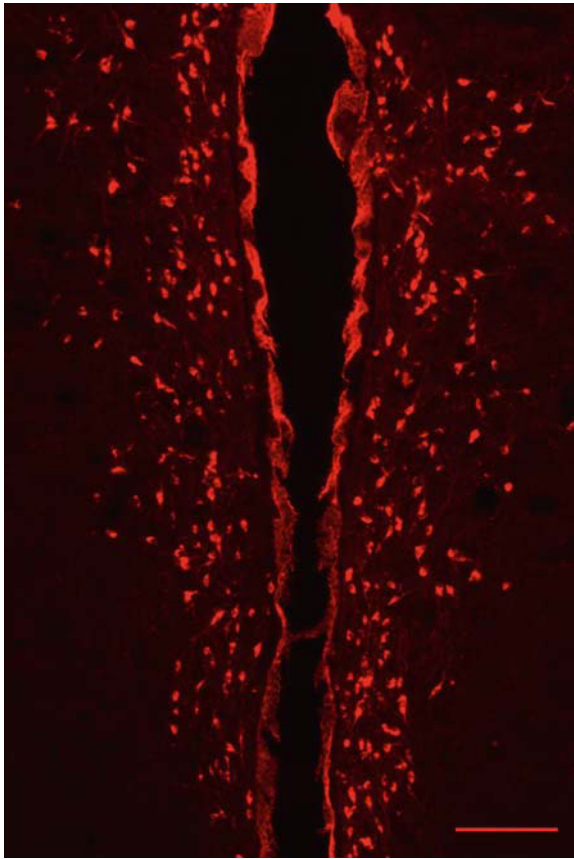
regulatory domains such as a cAMP ►response element (CRE). The intracellular mediator cAMP is one of the activators of somatostatin gene transcription, but also many other factors such as Ca²⁺, glucocorticoids and growth hormone are able to influence somatostatin gene expression [1].

As other neuropeptides, somatostatin is synthesised as a preprohormone on ribosomes of the rough ►endoplasmic reticulum (RER). Translation product of the 351 bp long mRNA coding sequence is preprosomatostatin, a peptide of 116 amino acids. After translocation of the precursor molecule into the ER lumen and cleavage of the signal peptide the prohormone is further transported by transfer vesicles through the Golgi stacks into the *trans* compartment of the ►golgi apparatus. Differential posttranslational processing of prosomatostatin on the way from the ER to the *trans* Golgi network results in two biologically active isoforms, the tetradecapeptide somatostatin-14 and the aminotermally extended octacosapeptide somatostatin-28, respectively. Cleavage of the C-terminal region of prosomatostatin at a dibasic Arg-Lys site produces somatostatin-14 and cleavage at a monobasic Gln-Arg site results in somatostatin-28. Candidates for somatostatin-14 converting enzymes are prohormone convertases PC1 and PC2 whereas ►furin is a candidate somatostatin-28 convertase. The amounts of produced isoforms are tissue specific. Whereas the hypothalamus synthesises both, somatostatin-14 and somatostatin-28 in a ratio of 4:1, the intestinal mucosal cells produce mainly somatostatin-28.

In the *trans* Golgi compartment the peptide is sorted via secretory vesicles into a regulated pathway. In contrast to vesicles of the constitutive pathway, vesicles of the regulated pathway are stored in the cytoplasm until their stimulated release. Membrane depolarisation as well as a wide range of substances such as intracellular mediators, neuropeptides, neurotransmitters, classical hormones, cytokines, growth factors, ions and nutrients induce somatostatin secretion. General inhibitors of somatostatin release are opiates, GABA, leptin and TGF-β.

Tissue Distribution

High amounts of somatostatin are found in the CNS, the peripheral nervous system, the gut and the endocrine pancreas whereas the kidneys, adrenals, thyroid, submandibular glands, prostate and placenta produce rather low amounts. In particular, the hypothalamus, all limbic structures, the deeper layers of the cerebral cortex, the striatum, the periaqueductal central grey and all levels of the major sensory pathway are brain areas that are especially rich in somatostatin. Eighty percent of the somatostatin immunoreactivity in the hypothalamus is found in cells of the anterior periventricular nucleus (Fig. 1, [1]). The gut δ cells of the mucosa and neurons, which are intrinsic to the submucous and



Somatostatin. Figure 1 Somatostatin-like immunoreactivity in neurons of the periventricular hypothalamic nucleus of the rat. Coronal brain cryostat sections have been processed for immunohistochemistry and sequentially incubated with a primary monoclonal mouse antihuman somatostatin antibody and secondary antimouse antibody conjugated with the fluorescence-dye Cy-3. Images have been taken with a Zeiss Axioplan fluorescence microscope. Scale bar, 100 μ m.

myenteric plexuses, produce somatostatin. Furthermore, somatostatin has been found in δ cells of the pancreas and within the thyroid where somatostatin has been detected in a subpopulation of C cells that additionally contain calcitonin. Somatostatin has also been localised in the inner part of the retina and in cells and organs of the immune system, for example within macrophages, lymphocytes and the thymus.

Somatostatin Receptors

Somatostatin acts on various organs, tissues and cells as neurotransmitter, paracrine/autocrine and endocrine regulator on cell secretion, smooth muscle contractility, nutrient absorption, cell growth and neurotransmission [1]. Some of its mainly inhibitory effects are listed in Table 1. Somatostatin mediates its function via a family of heptahelical G-protein-coupled receptors termed

*sst*₁, *sst*₂, *sst*₃, *sst*₄ and *sst*₅, which were cloned about 15 years ago. Despite a high degree of sequence homology the receptors derive from separate genes localised on different chromosomes (Table 2). The genes of *sst*₁, *sst*₃, *sst*₄ and *sst*₅ do not contain any introns in their protein coding regions. In contrast to this, the *sst*₂ gene contains a cryptic splice site at the 3' end of its coding region giving rise to two splice variants, *sst*_{2A} and *sst*_{2B}.

Studies investigating the distribution of *sst* mRNAs have shown that *sst* gene expression varies during ontogeny. Moreover, all five genes are tissue specifically expressed. However, expression pattern overlap and different mRNA levels have been demonstrated in brain, pituitary, pancreas, adrenals, kidneys, liver, lung, placenta, stomach, gut, thyroid and immune cells. In addition to the *sst* gene expression in organs, many tumour cell lines, such as AtT20 and GH3 pituitary cells, and numerous human tumours, benign or malignant, have been shown to be a rich source of *sst* subtypes. *sst*₂ mRNA is highly expressed in many tumours, while *sst*₅ mRNA is abundant in breast tumours and *sst*₁ appears to be preferentially expressed in primary prostate cancers [1, 3]. Laboratories investigating neuroendocrine tumours, such as gastrinomas and insulinomas, reported not only a general expression of *sst*₁, *sst*₂, but also varying levels of *sst*₃, *sst*₄ and *sst*₅ mRNAs have been observed. Interestingly, in gastrinomas and carcinoids only low amounts of *sst*₃ mRNA have been detected so far. However, it should be noted that these results may not automatically mirror functional receptor levels, since to date nearly all investigations of *sst* subtypes in tumours are based on mRNA studies.

Besides developmental and tissue-specific regulation of *sst* gene expression, regulation by extracellular signals, such as estrogen and thyroid hormone, has been observed. The underlying mechanisms still remain to be elucidated, although promoter studies of various laboratories have begun to work out the molecular basis for a better understanding. All *sst* subtype promoters investigated to date contain consensus sequences for several common transcription factors. For example, the *sst*₂ gene contains estrogen response elements whereas progesterone/glucocorticoid and thyroid response elements were found in the *sst*₁ and *sst*₅ genes [1].

The human *sst* receptor protein isoforms range in size from 364 amino acids to 418 amino acids (Table 2). The *sst* subtypes show highest sequence identity in their putative transmembrane domains and can be divided, on the basis of amino acid homologies and by their ability to bind somatostatin analogues (Table 3), into two subclasses, termed SRIF₁ and SRIF₂. The SRIF₁ group comprises *sst*₂, *sst*₃ and *sst*₅ (SRIF_{1A}, SRIF_{1B} and srif_{1C}) and the SRIF₂ group includes *sst*₁ and *sst*₄ (SRIF_{2A} and srif_{2B}). Receptors of the SRIF₁ group bind

Somatostatin. Table 1 Somatostatin effects in different tissues (Data adapted from [1, 2])

Site	Effects
Hypothalamus	Inhibition of norepinephrine, GHRH, TRH and CRH release
	Inhibition of endogenous SST release
	Effects on leptin signalling
Other brain regions	Effects on cognitive, locomotor, sensory and autonomic functions, analgesic effects
	Inhibition of dopamine release from the midbrain
	Stimulation of dopamine release in basal ganglia
Pituitary	Inhibition of basal and stimulated release of GH
	Inhibition of TSH release
	No effects on LH, FSH release
	No effect on ACTH release in normal subjects, but suppresses elevated levels in Addison's disease and in ACTH producing tumours
	No effect on prolactin release in normal subjects, but diminishes increased prolactin levels in acromegaly
Gastrointestinal tract	Inhibition of most gut hormones, gastric acid, pepsin, bile and colonic fluid secretion
	Suppression of motor activity in general, inhibition of gallbladder contraction, gastric emptying
	Stimulation of migrating motor complex activity
Thyroid	inhibition of TSH-stimulated T4 and T3 release
	Inhibition of calcitonin secretion from thyroid parafollicular cells
Adrenal	Inhibition of angiotensin II stimulated aldosterone release
	Inhibition of acetylcholine stimulated medullary catecholamine release
Kidney	Inhibition of hypovolemia stimulated renin secretion
	Inhibition of ADH-mediated water absorption
Immune cells	Diminishing IFN- γ secretion from lymphocytes
Lymphocytes, inflammatory cells, intestinal mucosal cells, cartilage cells and bone precursor cells	Inhibition of proliferation
Other tissues/cells	Inhibition of growth factor (IGF1, EGF, PDGF) and cytokine (IL6, IFN- γ) secretion

seglitide and octreotide with high (sst₂ and sst₅) to moderate (sst₃) affinity while members of the SRIF₂ group are insensitive to these compounds.

All six receptors seem to couple to ►**pertussis toxin-sensitive ►G proteins** of the Gi/Go type [1]. Depending on the sst subtype, cell type, and species different G-proteins couple the individual receptor isoforms to various second-messenger systems which include ►**adenylyl cyclase**, K⁺ and Ca²⁺ channels, Na⁺/H⁺ exchanger, ►**phospholipase C**, phospholipase A2, mitogen activated protein kinase, serine–threonine phosphatase, and phosphotyrosine phosphatase (Table 2). The fact that sst subtypes share transduction mechanisms, bind their endogenous ligands with nanomolar affinities, and that more than one receptor isoform can be expressed in a single cell, might indicate a functional interaction between the different receptor isoforms. In this respect

it should be noted that heterodimerisation of sst subtypes has been reported.

Physiological functions for sst subtypes have not yet been unequivocally recognised. It appears, however, that sst₂ predominantly mediates inhibition of glucagon release from pancreatic alpha-cells whereas sst₁ and sst₅ seem to be important regulators of insulin secretion and glucose regulation. For example, sst₁ or sst₅ knockout mice develop diabetes, and double-gene ablation of both receptors results in a distinct phenotype with hyperinsulinemia, islet cell hyperplasia and improved glucose tolerance. Furthermore, it is thought that sst₂ contributes to the regulation of gastric acid release and that sst₁, sst₂ and sst₅ inhibit hormone-stimulated and/or basal level secretion of GH secretion from the pituitary [1, 2]. Knockout mice have also helped to elucidate a role for sst₁ and sst₂ receptors in retinal functions [5].

Somatostatin. Table 2 Properties of sst subtypes (Data are adapted from [1, 2])

Receptor type	sst ₁ /SRIF _{2A}	sst ₂ /SRIF _{1A}	sst ₃ /srif _{1C}	sst ₄ /srif _{2B}	sst ₅ /SRIF _{1B}
Chromosomal localisation	14q13	17q24	22p13.1	20p11.2	16p13.3
Length of human receptor	391 aa	369 aa (sst _{2A})	418 aa	388 aa	364 aa
		356 aa (sst _{2B})			
Transduction mechanism	Inhibition of adenylyl cyclase; stimulation of tyrosine phosphatase activity; stimulation of MAP kinase activity; activation of ERK; inhibition of Ca ²⁺ channel activation; stimulation of Na ⁺ /H ⁺ exchanger; stimulation of AMPA/kainate glutamate channels	Inhibition of forskolin-stimulated adenylyl cyclase; activation of phosphoinositide metabolism; stimulation of tyrosine phosphatase activity; inhibition of Ca ²⁺ channel activation; activation of K ⁺ channel; inhibition of AMPA/kainate glutamate channels; inhibition of MAP kinase activity; inhibition of ERK; stimulation of SHP-1 and SHP-2	Inhibition of adenylyl cyclase; stimulation of phosphoinositide metabolism; stimulation of tyrosine phosphatase; activation of K ⁺ channel; inhibition/stimulation of MAP kinase activity; induction of p53 and Bax	Inhibition of adenylyl cyclase; stimulation of MAP kinase; stimulation of p38; activation of tyrosine phosphatase; stimulation of K ⁺ channels and phospholipase A ₂	Inhibition of adenylyl cyclase; activation/inhibition of phosphoinositide metabolism; inhibition of Ca ²⁺ influx; activation of K ⁺ channels; inhibition of MAP kinase; stimulation of tyrosine phosphatase

Somatostatin Related Peptides

Natural binding partners of all sst subtypes cloned to date are somatostatin-14 and somatostatin-28, which are bound with nanomolar affinity. sst₁₋₄ bind somatostatin-14 with comparable affinities, while sst₅ may have a slight preference for somatostatin-28. Cortistatin, a recently discovered closely related peptide, also binds sst subtypes with high affinity *in vitro*. Initially it was thought that it is mainly restricted to the cerebral cortex and the hippocampus, but recent studies have shown that cortistatin mRNA can also be obtained from peripheral tissues and tumour cells. Cortistatin shows functional characteristics that have not been demonstrated for somatostatin, such as sleep modulating properties. So far a cognate cortistatin receptor has not been identified, although an orphan receptor called MrgX2 has been shown to bind cortistatin, but not somatostatin, with high affinity *in vitro*. However, the MrgX2 mRNA expression pattern is not entirely consistent with the pattern expected for a cortistatin receptor. In addition, cortistatin binds to the ghrelin receptor GHSR1a with similar affinity than ghrelin [1].

Drugs

The diverse effects of somatostatin, such as inhibition of cell proliferation and hormone release, led to the suggestion that it could be used for the treatment of various diseases. However, its short plasma half-life (less than 3 min) and its relative low receptor subtype

selectivity make it less useful for therapeutic purposes. Therefore, several synthetic analogues (Table 3) with a higher stability to enzymatic degradation and a higher selectivity for specific sst subtypes have been synthesised. The synthetic peptide analogues have a decisive amino acid motif in common, since pharmacological studies have shown, that the amino acid residues 7–10 (Phe⁷-Trp⁸-Lys⁹-Thr¹⁰) of somatostatin are the responsible segment for receptor binding. In particular Trp⁸ and Lys⁹ are essential, while the remaining two amino acids may be replaced. Phe⁷ can be exchanged with tyrosine and Thr¹⁰ can be substituted by serine or valine. To date, only a few potential antagonists, such as cyanamid 154806, sst₃-ODN-8 and BIM-23056 are available, which preferentially bind to human sst₂, sst₃ and sst₅, respectively [1, 2, 4].

Today three analogues, octreotide, lanreotide and vapreotide are clinically used. All three substances display high affinity for sst₂ and sst₅. Already in the 1980s octreotide was used in the therapy of acromegaly and other neuroendocrine tumours. Acromegaly is a chronic disease of growth hormone (GH) hypersecretion and in most cases is caused by a pituitary adenoma. Somatostatin analogues have been shown to improve the clinical symptoms of acromegaly, for example gigantism in children, disfigurements of the hands, feet and the face in adults, headache and perspiration. Moreover, tumour shrinkage has been observed in about 50% of the patients, even though the tumour size reduction is reversible. The

Somatostatin. Table 3 Selected somatostatin analogues

sst Subtype ligands and analogues	Binding properties
<i>Endogenous Ligands</i>	
Somatostatin-14	Binding to all sst subtypes with high affinity
Somatostatin-28	Binding to all sst subtypes with high affinity, but slightly higher preference for sst ₅
Human cortistatin-17	Binding to sst ₂₋₅ with high affinity, slightly lower preference for sst ₁
Rat cortistatin-29	Binding to sst ₃ with high affinity, slightly lower preference for sst ₁ , sst ₄ and sst ₂ , and lower preference for sst ₅ (i.e. ~100-fold lower than that of somatostatin-14)
<i>Synthetic peptide analogues</i>	
Octreotide (SMS201–995)	Binding to sst ₂ with high affinity, slightly lower affinity to sst ₅ and sst ₃
Vapreotide (RC-160)	Binding to sst ₅ and sst ₂ with high affinity, moderate affinity for sst ₃ and sst ₄
Lanreotide (BIM23014)	Binding to sst ₂ and sst ₅ with high affinity, moderate affinity for sst ₃ (and sst ₄).
Seglitide (MK678)	Binding to sst ₂ and sst ₅ with high affinity, moderate affinity for sst ₃
BIM23268	Binding to sst ₅ with high affinity, slightly lower preference for sst ₂ , sst ₄ and sst ₁ , moderate affinity for sst ₃
NC8–12	Binding to sst ₃ and sst ₂ with very high affinity
BIM23197	Binding to sst ₂ and sst ₅ with high affinity, moderate affinity for sst ₃
CH275	Binding to sst ₁ and sst ₄ with high affinity
SOM230	Binding to sst ₁ , sst ₂ , sst ₃ and sst ₅ with high affinity, lower affinity to sst ₄
<i>Nonpeptide agonists</i>	
L-797,591	Binding to sst ₁ and sst ₂ with high affinity
L-779,976	Binding to sst ₂ with very high affinity
L-796,778	Binding to sst ₃ with moderate affinity.
L-803,087	Binding to sst ₄ with high affinity
L-817,818	Binding to sst ₅ with high affinity, with slightly lower affinity to sst ₁ , moderate affinities for sst ₂ , sst ₃ and sst ₄
<i>Radioligands</i>	
¹²³ I-Tyr ³ -octreotide	Binding to sst ₂ and sst ₅ with high affinity, lower affinity to sst ₃
¹¹¹ In-DTPA-D-Phe ¹ -octreotide ^a	Binding to sst ₂ and sst ₅ with high affinity, lower affinity to sst ₃
¹¹¹ In/ ⁹⁰ Y-DOTA-lanreotide ^b	Binding to sst ₂ , sst ₃ , sst ₄ and sst ₅ with high affinity, lower affinity to sst ₁
¹¹¹ In-DOTA-D-Phe ¹ -Tyr ³ -octreotide	Binding to sst ₂ and sst ₅ with high affinity, lower affinity to sst ₃
^{99m} Tc-depreotide	Binding to sst ₂ , sst ₃ and sst ₅ with high affinity
¹¹¹ In-DTPA-Tyr ³ -octreotate	Binding to sst ₂ and sst ₅ with high affinity
<i>Antagonists</i>	
BIM23056	sst ₅ Selective
sst ₃ -ODN-8	sst ₃ Selective
Cyanamid 154806	sst ₂ Selective
BIM23627	sst ₂ Selective
<i>Dopastatins</i>	
BIM23A387	Binding to sst ₂ and dopamine receptor D2DR with high affinity
BIM23A761	Binding to sst ₂ , sst ₅ and dopamine receptor D2DR with high affinity
BIM23A765	Binding to sst ₂ , sst ₅ and dopamine receptor D2DR with high affinity

Data are adapted from [1–4].

^aDTPA, diethylenetriaminepentaacetic acid.

^bDOTA, 1,4,7,10-tetraazacyclododecane-N,N',N',N'-tetraacetic acid.

decreased tumour volume is presumably caused by a shrinkage of individual tumour cells. Recently, sustained release formulations of octreotide and lanreotide have been developed. These new drugs are administered by intramuscular injections only every 7–28 days making life of patients easier, who need a long-term therapy. Furthermore, a novel sst analogue named SOM230 [4] that binds to sst_{1–3} and sst₅ with high affinity has been tested in a first trial in 12 acromegalic patients. The study showed that SOM230 was more effective in suppressing GH concentrations than octreotide. Thus, results of larger ongoing clinical trials testing this promising analogue are eagerly awaited.

Octreotide treatment achieves also good results in patients with insulinomas, gastrinomas, glucagonomas, ►VIPomas and metastatic carcinoids expressing sst₂ and sst₅. Symptoms such as peptic ulceration, diarrhoea, dehydration and necrolytic skin lesions rapidly improve giving the life of patients a higher quality. Despite the successful reduction of symptoms only in about 20% of the patients a tumour shrinkage was observed and, in contrast to acromegalic patients, most patients suffering from carcinoids became insensitive to octreotide therapy within weeks to months. The desensitisation might be explained by a *downregulation* of sst subtypes or more likely by an outgrowth of sst subtype-negative cell clones.

Other tumours, such as ACTH-secreting pituitary adenomas, prolactinomas, pancreatic and prostate cancer, which express no or other sst subtypes than sst₂ and sst₅ are rather unresponsive to octreotide therapy. Therefore, the development of new sst-subtype-selective analogues is required. Transfer of genes encoding sst₂ or sst₅ into such tumour types might also be a strategy, since clinically used somatostatin analogues exert the majority of their antineoplastic effects via these receptor subtypes. Furthermore, stable transfection of human pancreatic cancer cell lines with human sst₂ cDNA resulted in a significant reduction of tumour cell growth, indicating that the loss of sst₂ expression in pancreatic cancer could be responsible for the growth advantage of this kind of tumour [3, 4].

Somatostatin Receptor Scintigraphy and Receptor-Targeted Radiotherapy

Radiolabelled somatostatin analogues, such as ¹¹¹In-DTPA-D-Phe¹-octreotide or ¹¹¹In-DOTA-lanreotide are employed for somatostatin receptor scintigraphy (SRS), an imaging technique which nowadays is used in many hospitals to visualise sst subtype positive tumours in vivo. The high sensitivity of this method can be explained by an accumulation of radioligands within the tumours, which is presumably caused by the internalisation of the agonist-receptor complex [3]. Many human tumours seem to contain high mRNA levels for specific sst subtypes. For example, sst₂ is the

predominant isoform expressed by neuroendocrine tumours, whereas intestinal adenocarcinomas mostly contain sst₃ and sst₄. Therefore, in particular ¹¹¹In-DOTA-lanreotide is a very suitable tool for the detection of a wide range of tumours, since it binds not only sst₂ and sst₅ with high affinity but also sst₃ and sst₄ and to a lesser extent sst₁.

Furthermore, some of these radioligands have been used in first trials of receptor-targeted radiotherapy. Although ¹¹¹In is not the most favourable substance for radiotherapy, large amounts of ¹¹¹In-DTPA-D-Phe¹-octreotide were used for the treatment of patients suffering from neuroendocrine tumours. In a patient with an inoperable metastasised glucagonoma treatment with ¹¹¹In-DTPA-D-Phe¹-octreotide led to a small but significant reduction of tumour size and transiently lowered plasma glucagon levels [3]. Two further analogues, ⁹⁰Y-DOTA-lanreotide and ⁹⁰Y-DOTA-D-Phe¹-Tyr³-octreotide, have also been tested for tumour therapy. In a phase II study investigating more than 60 tumour patients treated with ⁹⁰Y-DOTA-lanreotide about 35% of the patients showed a stabilisation of tumour growth and in 15% of the cases a tumour regression was observed. However, clinical long-term trials are still needed to evaluate the therapeutic effectiveness of somatostatin analogues in cancer treatment.

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Somatotropin Release Inhibitory Factor

► Somatostatin

SOS

Synonyms

Son of seven less

Definition

A nucleotide exchange factor that activates Ras in response to external cues such as growth factors. Localizes to activated receptors, which contain intrinsic tyrosine kinases, through its interaction with the adaptor protein Grb-2.

► Adaptor Proteins

Spermidine

Spermidine is one of the most common natural polyamines with the following structure: $\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2$.

Chemical names:

N-(3-aminopropyl)butane-1,4-diamine

N-3-aminopropyl-1,4-diaminobutane

► Inwardly Rectifying K^+ Channels

► Polyamines

Spermine

Spermine is one of the most common natural polyamines in eukaryotes with the following structure: $\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2$.

Chemical names:

N,N'-bis(3-aminopropyl)butane-1,4-diamine

Bis(N-3-aminopropyl)-1,4-diaminobutane

► Inwardly Rectifying K^+ Channels

► Polyamines

Sphingosine Kinases

Sphingosine kinases (SphKs) catalyse the phosphorylation of sphingosine to sphingosine-1-phosphate (S1P).

Until now, two mammalian isoforms of SphK, SphK1 (43 kDa) and SphK2 (65 kDa) have been identified. Their catalytic domain is homologous to that of diacylglycerol kinases. SphK1 and SphK2 have distinct kinetic properties and are differentially expressed. SphK1 promotes cell growth and protects from apoptosis. It is upregulated in tumour cells and has features of an oncogene. SphK2, in contrast, mediates apoptosis. Deficiency of either SphK1 or SphK2 in mice does not cause obvious abnormalities, while double knockout mice die during embryogenesis. These data suggest that despite opposing functions the two sphingosine kinase isoenzymes can substitute for each other. SphK activity is regulated both transcriptionally and post-transcriptionally by GPCRs, receptor tyrosine kinases, cytokine and antigen receptors. However, SphK1 has a high basal activity and is probably mainly regulated by control of its subcellular localization. Several stimuli induce a rapid translocation of SphK1 from the cytosol to the plasma membrane. S1P that is formed in close vicinity to the membrane can then be extruded and act on S1P-GPCRs, a process named “inside-out signalling”. Examples are NGF-induced neurite extension in dorsal root ganglion cells (via S1P₁ and S1P₅) and antigen-induced degranulation of mast cells (via S1P₂). Another model suggests receptor signalling platforms rather than autocrine activation of S1P-GPCR, for example in platelet-derived growth factor-induced migration. Other stimuli, such as phosphatidic acid, induce a translocation of SphK1 to intracellular sites, supporting the view that S1P can also act as an intracellular mediator. Both SphK1 and SphK2 furthermore occur in the nucleus.

► Lysophospholipids

Sphingosine-1-phosphate

Sphingosine-1-phosphate (S1P) is a versatile bioactive lipid which can act as first and second messenger. Extracellular S1P is a *lysophospholipid* mediator and activates specific G-protein-coupled receptors (see: *lysophospholipids*). On the other hand, stimulation of many cells with diverse agonists causes a rapid and transient increase in intracellular S1P formation, and inhibition of this S1P formation by *sphingosine kinase* inhibitors abrogates agonist-induced Ca^{2+} mobilization. Approaches such as microinjection of S1P, or photolysis of caged S1P, support the hypothesis that intracellular S1P mediates mobilization of Ca^{2+} from intracellular stores. The sphingosine kinase/S1P signal transduction pathway may thus be a Ca^{2+} mobilization pathway in

addition to the well-characterized phospholipase C/inositol-1,4,5-trisphosphate pathway, however, intracellular target sites of SIP have not been identified so far.

► **Lysophospholipids**

Sphingosylphosphorylcholine

Synonyms

SPC

Definition

Signaling phospholipid derived from sphingomyelin by phospholipase action. Activates SIP receptors.

► **Proton-Sensing GPCRs**

Spike-ins

Spike-ins are usually RNA transcripts used to calibrate measurements in a DNA microarray experiment. Each spike-in is designed to hybridize with a specific control probe on the target array. Manufacturers of commercially available microarrays typically offer companion RNA spike-ins 'kits'. Known amounts of RNA spike-ins are mixed with the experiment sample during preparation. Subsequently the measured degree of hybridization between the spike-ins and the control probes is used to normalize the hybridization measurements of the sample RNA.

► **Gene Expression Analysis and Microarray Technology**

Spindle Assembly Checkpoint

A signal transduction pathway required for proper chromosome alignment during mitosis. The spindle assembly checkpoint is activated during mitosis in response to the presence of chromosomes that are not attached to spindle microtubules or that are not properly aligned at the metaphase plate. The spindle checkpoint

is turned off when all chromosomes are attached to microtubules and when a tension across kinetochores has been generated. The spindle checkpoint is essential for maintaining chromosomal stability.

► **Cell Cycle Control**

Splice Variant

A splice variant can arise when a gene contains at least two introns leading to the possibility that the DNA between them (an exon) may not be included in the final mRNA and protein product. Thus, the final protein product may exist in two forms: one containing the amino-acid sequence encoded by the exon that is located between the introns in the original DNA, and another form in which the amino-acid sequence encoded by that exon has been 'spliced out'. These two products are referred to as splice variants.

Splicing

Splicing is a processing step of the pre-mRNA to become a mature transcript. This involves the excision of intervening noncoding sequences (introns) from coding sequences (exons) by a multiple protein complex, the spliceosome. After splicing the mRNA molecule is ready for translation, since it contains a continuous sequence that encode an entire protein.

► **Transcriptional Regulation**

Sporotrichosis

Sporotrichosis is the fungal disease caused by *Sporotrix schenckii* and involves the lymphatic and subcutaneous tissues. The lesions spread via the lymphatics from the original wound and form nodules or pustules that quickly ulcerate. Dissemination is rare.

► **Antifungal Drugs**

SPRM

- Selective Progesterone Receptor Modulators

SR

- Sarcoplasmatic Reticulum

Src Gene

The c-src gene encodes the non-receptor tyrosine kinase pp60^{c-src} that is involved in signal transduction. The pp60^{c-src} protein consists of a tyrosine kinase domain, a src homology 2 (SH2) domain that can bind to receptor tyrosine kinases, and a src homology 3 (SH3) domain that binds to proline rich sequences. The viral homolog of c-src, v-src, from the Rous sarcoma virus is a potent oncogene.

- Tyrosin Kinases

Src-homology 2 Domain

The Src-homology 2 (SH2) domain is a protein domain of roughly 100 amino acids found in many signaling molecules. It binds to phosphorylated tyrosines, in particular peptide sequences on activated receptor tyrosine kinases or docking proteins. By recognizing specific phosphorylated tyrosines, these small domains serve as modules that enable the proteins that contain them to bind to activated receptor tyrosine kinases or other intracellular signaling proteins that have been transiently phosphorylated on tyrosines.

- PTB Domain
- Adaptor Proteins
- Phosphotyrosine-Binding (PTB) Domain
- Molecular Modeling
- Tyrosine Kinases

Src-homology 3 Domain

The Src homology 3 domain is a 60 amino acid long domain that binds to proline-rich sequences, thereby enabling protein-protein interactions.

- Growth Factors

Src Kinase

Synonyms

Sarcoma Kinase

Definition

The first tyrosine kinase discovered. A mammalian homologue of a viral protein that causes sarcoma. Important for cell-cell signaling and cell growth. The c-src gene encodes the non-receptor tyrosine kinase pp60^{c-src} that is involved in signal transduction. The pp60^{c-src} protein consists of a tyrosine kinase domain, a src homology 2 (SH2) domain that can bind to receptor tyrosine kinases, and a src homology 3 (SH3) domain that binds to proline rich sequences. The viral homolog of c-src, v-src, from the Rous sarcoma virus is a potent oncogene.

- Adaptor Proteins
- Tyrosin Kinases

SRP

- Signal Recognition Particle

SSRIs

- Selective Serotonin Reuptake Inhibitors
- Serotonergic System

SST14

- Somatostatin

SST28

- Somatostatin

STAT

Signal transducers and activators of transcription constitute a highly conserved family of proteins with the dual function of transducing signals from the cell surface into the nucleus as well as activating transcription of target genes. They convert extracellular stimuli into a wide range of appropriate cellular processes, such as immune response, antiviral protection, and proliferation.

- JAK-STAT-Pathway

State-dependent Block

State-dependent block describes the binding of a drug to a certain state of an ionic channel. Thus, the fast Na^+ channel switches between a resting and open and inactivated states, the latter being the state to which antiarrhythmic drugs like lidocaine bind.

- Antiarrhythmic Drugs

Statins

A class of cholesterol lowering drugs that inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting enzyme step in cholesterol biosynthesis.

- HMG-CoA-Reductase Inhibitors

Status Asthmaticus

An acute and life-threatening exacerbation of asthma that does not respond to standard treatments of bronchodilators and corticosteroids.

- Bronchial Asthma

Steroid Receptors

Steroid receptors belong to the nuclear receptor superfamily and bind steroid hormones. They are cytoplasmic when inactive and associated with chaperones.

- Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
- Gluco-mineralcorticoid Receptors

Steroids

Steroids are a group of natural substances which share a common basic structure consisting of four condensed rings (sterane). Cholesterol is the precursor for the group of steroid hormones which are mainly formed in the adrenal medulla and in the gonads, including androgens, progesterone and estrogens as well as the mineralocorticoids. Steroid Hormones are lipophilic substances which bind to intracellular receptors. Other important steroids include the bile acids and the D-vitamins. Many related substances are found in plants, often present as glycosides. An important example of plant steroids are the cardiac glycosides.

- Contraceptives
- Gluco-mineralcorticoid Receptor
- Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
- Glucocorticoids

Sterol Transporters

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Definition

Transport of sterols, in particular reverse cholesterol transport, plays a central role in the pathogenesis of atherosclerosis. In addition to almost inefficient passive diffusion, sterol transport to various acceptors is mediated by membrane-bound energy-dependent transporters. The so far best characterized transporters belong to the family of ATP-binding cassette (ABC) transporters (►ATP-binding cassette transporter superfamily) of which ABCA1, ABCG1, and ABCG5 and ABCG8 are well-known sterol transporters. Moreover, two further members of this family, ABCA7 and ABCB1 have been implicated to sterol transport.

Basic Characteristics

ABC-transporters are multispan membrane proteins that mediate the active uptake or efflux of specific substrates across various biological membrane systems. A functional ABC-transporter protein usually consists of two transmembrane domains and two ABCs. These cassettes consist of a Walker A and a Walker B motif, short conserved peptides, which are required for ATP binding. Between the two Walker motifs an additional element, the signature motif is localized, which is characteristic for each ABC subfamily. Genes of the ABC-transporter family either encode for full size transporter including already the two transmembrane domains and the two ABCs or, alternatively, encode for so called half-size transporters which have to assemble to homo- or heterodimers to constitute a fully functional transporter. The final localization of the full-size transporter is the plasma membrane, however, due to vesicular trafficking processes, they can also be found intracellularly. Depending on their mode of action ABC-transporters can be split into two different sections. The active transporters or pumps couple the hydrolysis of ATP and the resulting free energy to movement of molecules across membranes against a chemical concentration gradient. In contrast several other ABC proteins show nucleotide binding and a subsequent conformational change but very low ATP hydrolysis including ABCA1. To date, 48 human ABC-transporters, which are subdivided into seven subfamilies, have been identified and many of these proteins exert their biological activities in lipid metabolism and are themselves

regulated by lipids including phospholipids, sterols, and fatty acids.

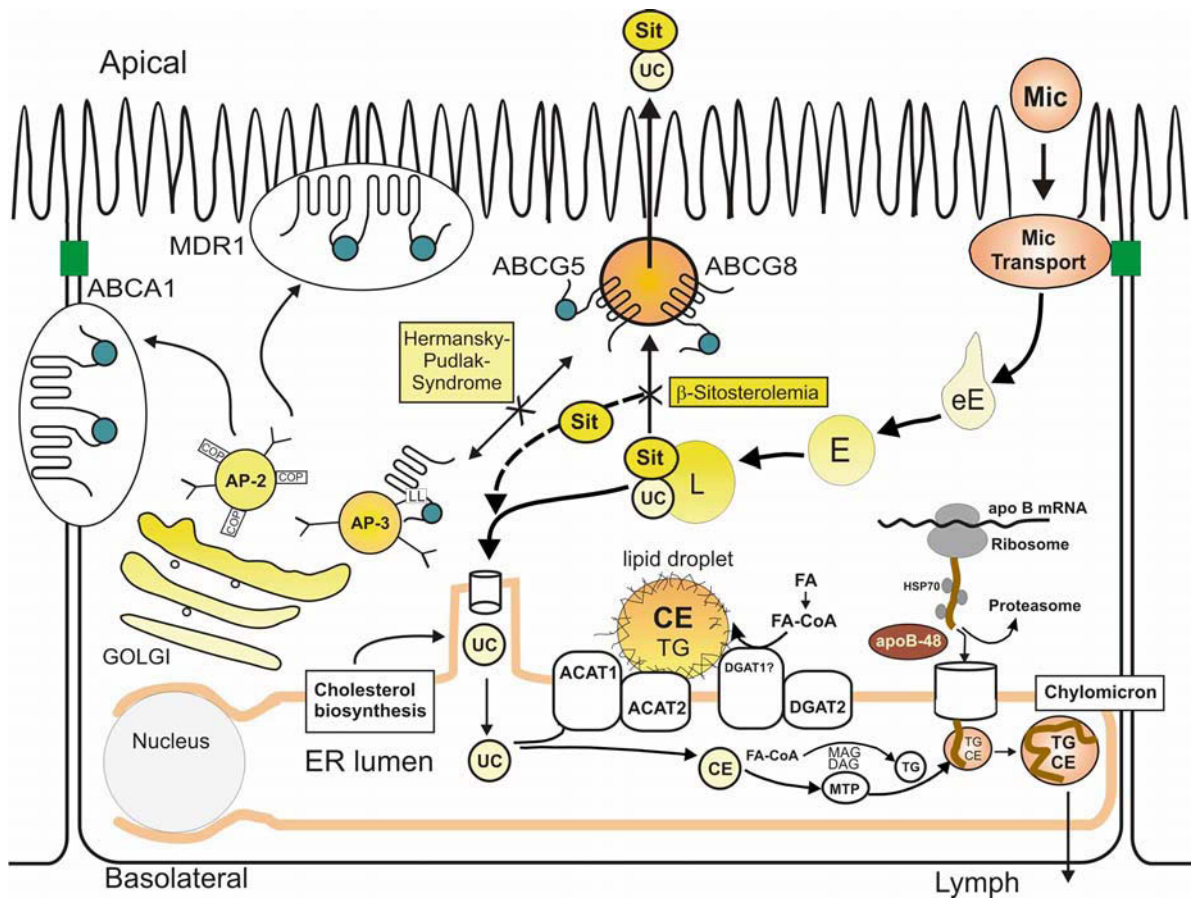
ABCA1

ABCA1 is the founding member of the ABCA-family and is still under extensive investigation. The *ABCA1* gene encodes a full size transporter and consists of 50 exons located on chromosome 9q31. The predominant role of ABCA1 is the regulation of cellular ►cholesterol and phospholipid efflux to cholesterol acceptors such as high-density lipoproteins (HDL) and lipid-poor apolipoproteins. This process may be indirect and ATP-dependent. ABCA1 expression is found in a variety of human organs such as placenta, liver, intestine (Fig 1), lung, and adrenal glands with very high expression levels in cholesterol loaded macrophages. The cholesterol induced transcription of ABCA1 is primarily mediated by the activation of the nuclear hormone receptor heterodimer liver X receptor (LXR) (►nuclear receptors) with oxysterols as naturally occurring ligand and retinoid X receptor RXR. In addition, ABCA1 expression is induced indirectly via LXR induction by peroxisome proliferator activated receptor γ (►PPAR γ) (PPARs). The ABCA1 promoter is further stimulated by the ubiquitous transcription factor Sp1 and the hypoxia-induced factor 1 (HIF1). Shutdown of ABCA1 expression in the absence of sterol or in certain tissues is mediated by corepressor complexes involving unliganded LXR, SREBP2, Sp3, and the SCAN-domain zinc finger transcription factor ZNF202, which also impacts nuclear signaling.

Mutations of the human ABCA1 gene are the direct cause of HDL-deficiency syndromes such as Tangier disease. In these patients the most striking feature is the almost complete absence of plasma HDL with low serum cholesterol levels and significantly reduced cellular efflux of cholesterol and phospholipids. The very low levels of plasma HDL observed in these patients are mainly caused by an enhanced catabolism of HDL precursors. The patients have high risk of premature atherosclerosis and coronary artery disease. Furthermore, these patients display accumulation of cholesteryl esters in the cells of the reticuloendothelial system resulting in splenomegaly and enlargement of lymph nodes and tonsils.

ABCA7

Based on experimental data the full-length transporter ABCA7 has been implicated in the cellular efflux of serine containing lipids such as phosphatidylserine and ceramide and maybe cholesterol. Novel data also demonstrate a role for ABCA7 in phagocytosis. Since ABCA7 knockout animals also display gender-dependent reduction in serum cholesterol levels as well as in visceral fat, strong evidence suggests a significant role for ABCA7 in sterol transport. The human *ABCA7* gene consists of 46 exons and, due to



Sterol Transporters. Figure 1 Proposed role of ABC proteins in intestinal sterol metabolism. ABCG 5, ABCG8 and ABCA1 are sterol-induced members of the ABC-transporter family. ABCG5 and ABCG8 mediate the export of plant sterols and cholesterol into the gut lumen. In contrast, ABCA1 expression and function are required for the uptake of sterols into intestinal epithelial cells. Implications for intracellular location and vesicular trafficking of these proteins are presented. Abbreviations not defined in the text: CE, cholesteryl ester; DAG, diacylglyceride; DGAT, acyl-CoA: diacylglycerol transferase; HSP70, heat shock protein 70; L, lysosome; MAG, monoacylglyceride; Mic, micelle; MTP, microsomal transfer protein; Sit, sitosterol (adapted from Schmitz and Langmann, 2003 [5]).

alternative splicing, two main ABCA7 isoforms, type I and type II, with distinct expression patterns do exist. While type I mRNA is higher expressed in brain and bone marrow, type II is predominantly found in spleen, thymus, lymph node, and trachea. The ABCA7 gene is located in close proximity to the gene for the minor histocompatibility antigen HA-1 on chromosome 19p13.3 in a head-to-tail array. Between both genes a functional and regulatory interlink might exist, which is especially interesting since ABCA7 has been shown to be regulated by sterols via SREBP2. In addition to its role in lipid metabolism ABCA7/HA-1 may be related to autoimmune disorders such as Sjögren's syndrome.

ABCB1

ABCB1 (also called MDR1) has initially been described by its ability to confer a multidrug resistance phenotype to cancer cells upon chemotherapy. This full-size

transporter is localized to the apical membrane of polarized cells (Fig 1) and the major sites of expression are found in liver, intestine, and the blood-brain barrier. One main physiological function of ABCB1 is the protection of cells by extruding lipophilic drugs. Indeed, ABCB1 is a highly promiscuous transporter of a variety of hydrophobic drugs including vinblastine, colchicines, VP16, and adriamycin. In addition, cholesterol and a number of different other lipids like phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and glucosylceramide are substrates for ABCB1-mediated transport. In addition to its role in drug and lipid transport, ABCB1 has also been related to inflammatory processes and immune response as well as to Alzheimer's disease, since it is able to translocate, e.g., bioactive lipids like platelet-activating factor and has been implicated in the in the efflux of brain β -amyloid protein.

ABCG1

The half-size transporter ABCG1 reveals a strikingly similar expression and regulation pattern to ABCA1. Like ABCA1, ABCG1 is upregulated during the differentiation process of monocytes to mature macrophages and is strongly induced by foam cell formation of these macrophages under sterol loading conditions, while cholesterol deloading of the cells with lipid acceptors such as ►HDL results in a downregulation of ABCG1 mRNA and protein expression. This regulation of ABCG1 exclusively occurs in human or murine monomyeloid cells, such as primary human macrophages, THP-1 cells, RAW246.7 cells, or foam cells from atherosclerotic lesions. This sterol-sensitive induction of ABCG1 is independent of proinflammatory stimuli and the oxidative state of the cell and is mediated by the LXR/RXR pathway. Similar to ABCA1, an additional inhibitory mechanism involving the transcription factor ZNF202 has been described. However, due to multiple alternative promoters transcriptional regulation of ABCG1 appears very complex and several different isoforms with obviously distinct tissue expression patterns do exist. Besides monomyeloid cells, ABCG1 is mainly expressed in spleen, thymus, lung, and brain.

The function of ABCG1 in lipid transport is not fully understood so far. In contrast to ABCA1, ABCG1 is not able to efflux lipids to lipid-poor lipoproteins, but requires more mature α -HDL subclasses as acceptor particles rather than pre- β -HDL or other lipid acceptors such as phosphatidylcholine vesicles. Since the activity of ABCA1 in lipidation of lipid-poor apolipoproteins is sufficient to generate an efficient acceptor for ABCG1-mediated cholesterol efflux, a tow-step model for ABCA1- and ABCG1-mediated cellular lipid efflux is proposed: Initially, ABCA1 may promote cholesterol and phospholipid efflux to lipid-poor Apo AI and ABCG1 subsequently promotes the efflux of additional cellular cholesterol to perform lipid-protein complexes.

Although mutations of the human ABCG1 gene have not been described so far, data from ABCG1 knockout mice recently gave novel insights into ABCG1 function in vivo. Targeted disruption of the ABCG1 gene caused massive deposition of neutral lipids and phospholipids in liver, lungs, and tissue macrophages further supporting a critical role of ABCG1 in lipid transport.

ABCG5 and ABCG8

In addition to the described lipid pathways mainly operative in macrophages, two further ABC-transporters, ABCG5 and ABCG8 have been implicated in the efflux of dietary sterols from intestinal cells back into the gut lumen and from liver to the bile duct (Fig 1). Both ABC-transporters form a functional heterodimer with highest expression levels in liver and intestine and are regulated

by a common bidirectional promoter. Therefore, ABCG5 and ABCG8 share common regulatory elements, in particular, the LXR/RXR heterodimer that mediates cholesterol-induced expression of both genes. Mutations in ABCG5 or ABCG8 lead to β -Sitosterolemia, also called phytosterolemia or shellfishsterolemia, a rare autosomal recessive disorder. The disease is characterized by enhanced trapping of cholesterol and other sterols, including plant and shellfish sterols, within the intestinal cells and the inability to concentrate these sterols in the bile. As a consequence, affected individuals have strongly increased levels of plant sterols, e.g., β -sitosterol, campesterol, stigmasterol, avenosterol, and 5 α -saturated stanols, whereas total sterol levels remain normal or just moderately elevated. Patients mainly suffer from tendon and tuberous xanthomas at early age and premature development of atherosclerosis and coronary heart disease.

In addition to the ABCG5/ABCG8 heterodimer, a specific transporter termed Niemann–Pick C1 like 1 (NPC1L1) as well as aminopeptidase N (CD13) were recently identified to play a critical role in intestinal absorption of cholesterol and plant sterols. Both genes are expressed predominantly in the gastrointestinal tract with peak expression levels in the small intestine. CD13 is localized in the brush border membrane and is the target of ezetimibe, a specific inhibitor of intestinal cholesterol absorption. Deep tubular invaginations connect CD13 to the NPC1L1 pathway that controls downstream cholesterol trafficking by directing exogenous sterols toward the late endosome. Thereby, CD13 and NPC1L1 act as gatekeepers for absorption of cholesterol and plant sterols from the lumen of the small intestine. Sterols that have entered the intestinal cell can then be directly transported back to the gut lumen by the ABCG5/ABCG8 complex by means by a sort of kick-back mechanism. Although, the function of CD13, NPC1L1 and ABCG5/ABCG8 is not fully understood, it is very likely that these proteins play a key role in the regulation of total cholesterol absorption.

Drugs

Although no drugs directly targeting the sterol transporters described above are available, the function of several lipid lowering drugs is associated with interaction with ABCA1.

Adenosin A_{2A} receptor antagonists are novel drugs for the prevention and treatment of vascular and tissue injuries. The antiatherogenic effects of ►adenosin are at least partially mediated by an induction of ABCA1 expression. The observed upregulation of ABCA1 following A_{2A} receptor ligation is mediated by cAMP/protein kinase A (PKA) potentially acting via phosphorylation of Sp1 and the ABCA1 promoter.

Niacin is a further very potent drug in increasing HDL-cholesterol levels and lowering plasma low-density

lipoprotein (LDL) cholesterol and triglycerides which has a significant effect on cholesterol transport in macrophages. ► **Niacin** is able to upregulate LXR-mediated transcription of ABCA1 via an activation of transcription and nuclear translocation of PPAR γ as well as to increase cellular cAMP levels. It is very likely that niacin mediates its effects by affecting several key genes of reverse cholesterol transport which underlie nuclear receptor and PKA signaling, including ABCA1 and ABCG1.

In addition to Niacin, **fibrates** are very effective in raising HDL-cholesterol and lowering triglycerides. In particular efficacy of gemfibrozil and bezafibrate in reducing cardiovascular morbidity, mortality and progression of atherosclerosis has been demonstrated. Fibrates are synthetic PPAR α ligands, which impact HDL and triglyceride metabolism by regulating the expression of important genes in reverse cholesterol transport and fatty acid metabolism including ABCA1 and ABCG1 as well as Apo AI, Apo AII, Apo CIII, and lipoprotein lipase.

► **Ca²⁺ channel blockers**, such as verapamil, are known to exert potent antihypertensive, antiarrhythmic, and antiatherosclerotic effects. In some reports an increase in HDL-cholesterol was attributed to verapamil treatment which may be mediated by a verapamil-induced increase in ABCA1 expression that is independent of oxysterols and LXR, but may be caused by direct interaction of verapamil with the ABCA1 promoter.

Ezetimibe is a novel selective cholesterol absorption inhibitor, which does not affect uptake of fatty acids, bile acids, fat soluble vitamins, or carotenoids. The molecular target for ezetimibe is in the intestinal brush border membrane of CD13. In clinical trials with hypercholesterolemic patients ezetimibe has been shown to be highly effective in inhibiting intestinal cholesterol absorption and reducing plasma LDL-cholesterol.

- ATP-dependent K⁺ Channel
- Fatty Acid Transporters
- Nuclear Receptors
- Niacin
- Ca²⁺ Channel Blockers
- PPAR γ

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STIM

An ER protein (though perhaps also expressed elsewhere) that senses the Ca²⁺ content of the ER and thereby regulates store-operated Ca²⁺ entry.

- IP₃ Receptors

Stimulant Drugs

- Psychostimulants

Store-operated Ca²⁺ Entry

A mechanism whereby depletion of intracellular Ca²⁺ stores leads directly to activation of a Ca²⁺ channel in the plasma membrane.

- IP₃ Receptors
- TRP Channels

Streptokinase

It is a single-chain glycoprotein (47 kDa) produced by *Streptococcus hemolyticus*. Streptokinase is not an enzyme, but can form a complex with human plasminogen, and via a conformational change in the plasminogen an active center appears and functions as a plasminogen activator. The complex converts a neighboring free plasminogen to plasmin.

- Fibrinolytics

Stress

Stress is a physical or a psychological stimulus followed by catecholamine and glucocorticoid hormones release that may lead to mental or physiological reactions supporting survival in the short time range yet potentially causing consequent illness such as anxious (escape) or depressive (withdrawal) behaviour at a longer time range.

Stress Proteins

- Chaperones

Structural Bioinformatics

- Molecular Modeling

Structural Genomics

The genome projects produce an enormous amount of sequence data that needs to be annotated in terms of molecular structure and biological function.

Structural genomics aims to use high-throughput structure determination and computational analysis to provide structures and/or 3D-models of every tractable protein. The intention is to determine as many protein structures as possible and to exploit the solved structures for the assignment of biological function to hypothetical proteins.

- Bioinformatics
- Molecular Modeling

Strychnine

The convulsant alkaloid strychnine from the Indian tree *Strychnos nux vomica* is highly toxic. Strychnine

poisoning causes hyperreflexia, increased muscle tone, convulsions, and finally death. Strychnine binds to the inhibitory glycine receptor with high affinity ($K_D \approx 10$ nM), efficiently displacing the neurotransmitter glycine. Glycine-displaceable binding of [3 H] strychnine is a highly specific probe for the glycine receptor.

- Glycine Receptors

STX

- Saxitoxin

Substance K

- Neurokinin A
- Tachykinins
- Neuroleptics

Substance P

Substance P is a member of a group of polypeptides known as neurokinins or tachykinins. It is thought to be the primary neurotransmitter for the transfer of sensory information from the periphery to the spinal cord and brain. Substance P as well as neurokinin NK₁ receptors has been detected in vagal afferent neurons in the area postrema, nucleus tractus solitarius and dorsal motor nucleus of the vagus. Substance P has been shown to increase the firing rate of neurons in the area postrema and nucleus tractus solitarius and to produce retching when applied directly to these areas in animal studies.

- Nociception
- Tachykinins
- NSAIDs
- Non-steroidal Anti-inflammatory Drugs
- NGF
- Nerve Growth Factor
- Neurotrophic Factors
- Pain and Nociception

Substance Use Disorders

► Drug Addiction/Dependence

Substantia Gelatinosa

The substantia gelatinosa is part of the dorsal horn of the spinal cord, also called “lamina II”. The substantia gelatinosa is made up almost exclusively of interneurons (both excitatory and inhibitory), some of which respond only to nociceptive inputs, while others respond also to non-noxious stimuli.

► Nociception

Substantia Nigra

- Nigrostriatal Tract/Pathway
- Anti-Parkinson Drugs

Sulfonylurea Receptor

Synonyms

SUR

Definition

Regulatory subunit of the ATP-dependent potassium channel. Forms heterodimers with the channel subunit Kir 6.2; the channel is presumably composed of 4 SUR/Kir 6.2 heterodimers. Two isoforms, SUR1 and SUR2, and two splicing variants, SUR2A and SUR2B, have been identified and characterized. SUR contains multiple transmembrane domains and two nucleotide binding folds. ATP and β -cytotropic agents bind to SUR1 and block the channel; ADP produces the opposite effect. Loss of SUR or of the whole channel results in spontaneous depolarization and loss of the effect of ADP.

- Diabetes mellitus
- ATP-dependent K^+ Channels
- Antidiabetic Drugs Other than Insulin

Sulfonylureas

Sulfonylureas are oral hypoglycemic drugs widely used in treatment of type2 diabetes. The first generation of sulfonylureas includes tolbutamide, acetohexamide, and chlorpropamide. The second includes glibenclamide, gliclazide, and glimepiride, which are considerably more potent than the earlier agents.

- ATP-dependent K^+ Channels
- Antidiabetic Drugs Other than Insulin

Sulfotransferase

Sulfotransferases are a group of cytosolic enzymes, which catalyze the transfer of the sulfonate group from 3'-phosphoadenosine-5'-phosphosulfate (PRPS) to an acceptor substrate to form either a sulfate or sulfonate conjugate. Sulfotransferases metabolize a variety of small lipophilic endobiotics (e.g. estrogen, corticoid, thyroxine) as well as a variety of xenobiotics. Genes of cytosolic sulfotransferases (SULT) have been identified and several genetic polymorphisms have been observed in human sulfotransferases, such as ST1A2, ST1A3 and ST2A3.

SUMO

Small ubiquitin-like modifier represents a family of evolutionary conserved proteins that are distantly related in amino-acid sequence to ubiquitin, but share the same structural folding with ubiquitin proteins. SUMO proteins are covalently conjugated to protein substrates by an isopeptide bond through their carboxyl termini. SUMO addition to lysine residues of target proteins, termed SUMOylation, mediates post-translational modification and requires a set of enzymes that are distinct from those that act on ubiquitin. SUMOylation regulates the activity of a variety of target proteins including transcription factors.

- PIAS Proteins
- Ubiquitin/Proteasome
- SUMOylation

SUMOylation

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Synonyms

SUMO1 (Small Ubiquitin-like modifier 1); Sentrin; SMT3C (suppressor of MIF2 mutations 3); GMP1 (GAP-Modifying Protein 1); Ubl1 (Ubiquitin-like protein 1); PIC1 (PML interacting protein 1)

Definition

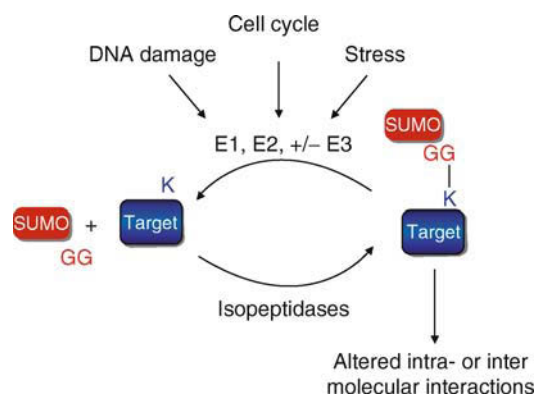
► **Small Ubiquitin-like modifier** (SUMO) is a conserved protein that is ubiquitously expressed in eukaryotes and is essential for viability. It serves as a reversible posttranslational modifier by forming an ► **isopeptide bond** with lysine residues in many target proteins, in a catalytic process termed SUMOylation. SUMOylation of proteins results in altered inter- or intramolecular interactions of the modified target (Fig. 1).

Basic Mechanisms

The SUMO Family

SUMO proteins belong to a family of posttranslational protein modifiers (Ubl for ubiquitin-like) of which ubiquitin is the founding member. Common to these proteins is the ubiquitin fold, an approximately 9 kDa globular core that consists of a $\beta\alpha\beta\beta\alpha\beta$ fold, and a GlyGly motif at the C-terminus of the mature Ubl. Like all other UbIs, SUMO proteins are translated as precursors that are processed before they are conjugated to their targets (see below). A characteristic feature of the SUMO family is a flexible amino terminus of 15–25 amino acids. The number of SUMO paralogs expressed in eukaryotic organisms varies from one (yeast) to eight (plants).

The vertebrate genome encodes two subfamilies of SUMO proteins, the first consists of SUMO1, and the second includes SUMO2, SUMO3, and SUMO4. The mature forms of SUMO2 and SUMO3 are 96% identical, but share only 45% identity with SUMO1. SUMO4 shares 85% identity with SUMO2 and -3; however, it is currently unclear whether this homolog is processed to a mature form and conjugated to substrates. SUMO1, -2, and -3 are expressed in all tissues, but vary in their cellular localization, target specificity, and function. While SUMO1 is found mostly conjugated to substrates, SUMO2/3 are found predominantly in the unconjugated form under normal growth conditions. Most targets are modified by attachment of one or a few single SUMO entities. In some cases, however, chains of SUMO2/3 are formed



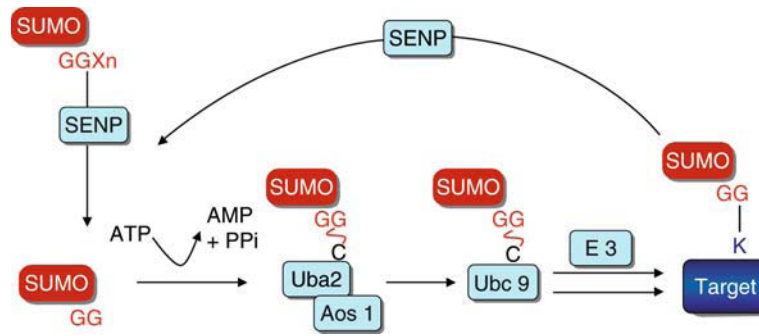
SUMOylation. Figure 1 SUMOylation is a reversible and regulated process. Target protein modification by SUMO can be initiated and terminated by different cues. Sumoylation leads to changes in the behavior of the modified protein, for example, different cellular localization, enhanced/reduced activity, or increased stability. These changes are due to alterations either in protein interactions or protein folding.

on the target. The function of these chains is largely unknown.

The Enzymatic Process

Proteins are conjugated to SUMO via an ► **isopeptide bond** formed between an exposed carboxy terminal glycine residue of SUMO and the ϵ -amino group of a lysine residue of the target protein. In most cases, the modified lysines in target proteins are found in a consensus SUMOylation site: ψ KxE/D, in which ψ is a large branched hydrophobic residue, and X is any amino acid. SUMOylation occurs if the motif is exposed and in an unstructured region. The reaction is carried out by at least two enzymes that act on all SUMO proteins: the E1 SUMO activating enzyme, a heterodimer consisting of Aos1 (SAE1) and Uba2 (SAE2), and the E2 enzyme also known as Ubc9 (Fig. 2).

In the first step, the E1 enzyme activates SUMO in an ATP-dependent manner. This reaction generates a high-energy thiol ester intermediate between the glycine residue at the C-terminus of SUMO and the active site cysteine of Uba2. Subsequently, SUMO is transferred from the E1 to a cysteine in the active site of Ubc9. In some cases, Aos1/Uba2 and Ubc9 are sufficient to modify a target. This is the case, for example, for RanGAP1, which interacts with Ubc9 by virtue of two binding interfaces, the SUMO consensus motif and an additional interface at its C-terminus. Most targets interact with Ubc9 only via their SUMOylation site and can be sumoylated efficiently only in the presence of additional factors, so called ► **E3 ligases**. SUMO E3 ligases function by stabilizing the Ubc9-target interaction and/or by accelerating transfer rates. Three types of E3 enzymes are known to act in SUMOylation.



SUMOylation. Figure 2 SUMO enzymatic machinery. SUMO is translated as a precursor with a short C-terminal prosequence. SUMO specific isopeptidases (SENPs) remove this sequence to reveal a double Glycine motif in SUMO that is essential for conjugation. The E1 heterodimer Uba2/Aos1 activates SUMO in an ATP-dependent reaction by forming a thioester bond between the C-terminal Glycine in SUMO and the active site Cysteine of the E1. SUMO is transferred from the E1 to the Cysteine of Ubc9. In most cases an E3 facilitates the transfer of SUMO from Ubc9 to the substrate (bold arrow). In rare cases Ubc9 can transfer SUMO directly to the substrate (broken arrow). As a result, a covalent bond is formed between the C-terminal Glycine of SUMO and a Lysine in the target protein. Finally, this modification can be reversed by SENPs.

The first family of SUMO E3 ligases is present in all eukaryotic organisms and is characterized by a **SP-RING finger domain** that mediates the interactions of the SUMO E2 enzyme Ubc9 with the substrate. The SP-RING is related to RING finger domains found in most ubiquitin E3 ligases. The group of vertebrate SP-RING finger E3 ligases includes PIAS proteins (PIAS1, PIAS3, PIASx α , PIASx β , PIASy and Mms21. Interestingly, PIAS proteins (*Protein inhibitor of activated STAT*) were initially identified as proteins that interact with and inhibit the STAT transcription factors (signal transducer and activator of transcription), or modulate transcriptional activity of the androgen receptor (shown for PIASx α also known as ARIP3). The second SUMO E3 ligase family is apparently vertebrate specific. It consists of the nuclear pore protein RanBP2/Nup358 that faces the cytosolic side of the nuclear envelope. The minimal E3 ligase activity of RanBP2 was mapped to an unfolded area of 120 amino acids in its C-terminus that does not resemble other known E3 ligases. Other domains of RanBP2 serve as binding sites for nuclear transport factors. Whether SUMOylation and nucleocytoplasmic transport are coupled events remains to be seen.

The third type of E3 ligases is represented by the polycomb protein 2 (Pc2), which was reported to enhance sumoylation of the substrate CtBP. N- and C-terminal domains in Pc2 that have been implicated in CtBP sumoylation do not resemble known E3 ligases. Like RanBP2, Pc2 expression is restricted to higher eukaryotes.

Modification by SUMO is a reversible and often highly dynamic process. Cleavage of the isopeptide bond between SUMO and its targets is accomplished by SUMO specific **cysteine proteases** of the Ulp/SENp family (Fig. 2). Six members were identified in humans,

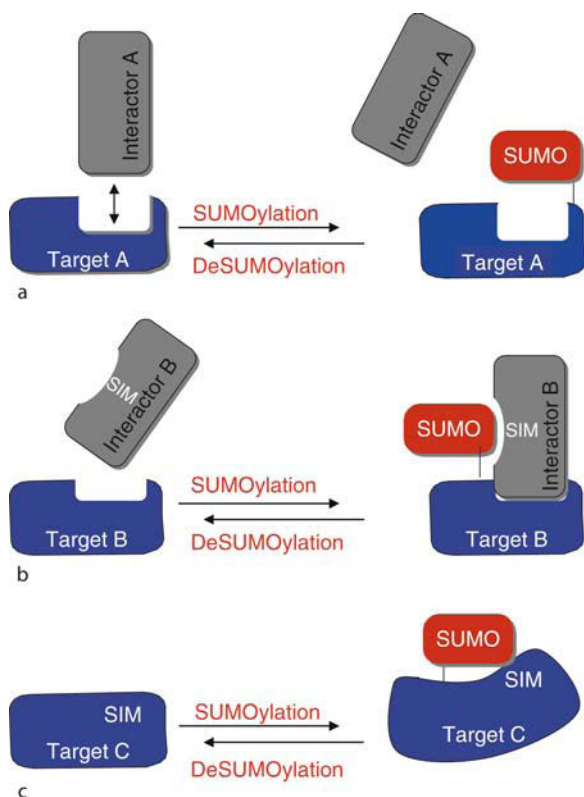
SENp1–3, and SENp5–7. They all share a 30 kDa catalytic domain at their C-terminus, but differ greatly in size and sequence. SENp3, -5, and -7 reside in the nucleus, SENp1 appears to shuttle between the cytoplasm and nucleus, SENp2 is at the nuclear pore complex, facing the nucleoplasm, and SENp6 is in the cytoplasm. SUMO isopeptidases also differ in their specificity against different SUMO-proteins (e.g., SENp3 and -5 preferentially deconjugate SUMO2/3) and may display selectivity toward modified targets. Due to these differences in activity and different localization, SENp proteases are believed to have distinct functions in vivo.

In addition SENPs are required for maturation of SUMO precursors. SUMO proteins are translated as inactive precursors with a short C-terminal prosequence of variable length. This sequence needs to be removed to expose a double glycine motif at the C-terminus of SUMO that is required for conjugation.

Molecular Consequences of SUMOylation

What does SUMOylation do? The answer to this question is almost as long as the number of substrates to which SUMO is conjugated. Protein modification by SUMO can lead for example to altered localization, activity, or stability of the target proteins. Although downstream consequences of sumoylation are target specific and highly diverse, the general feature underlying all effects is a change in inter- or intramolecular interactions (Fig. 3) SUMOylation leads to such changes by masking binding sites, formation of a new binding interface, or by inducing conformational changes in protein structure.

Masking of a site: Association between two proteins can be disrupted by SUMOylation, if the SUMO attachment site of a target protein overlaps with the



SUMOylation. Figure 3 SUMOylation affects protein–protein interaction. (a) Masking of an interaction site: The interaction between Target A and a second protein (interactor A) is hindered by SUMOylation, as the modification site is in close proximity to the interaction surface. (b) Formation of a novel site: Interacting protein B contains a weak binding site to Target B and a SUMO interacting motif (SIM). The association between Target B and interactor B is initiated/stabilized upon sumoylation of Target B, since it now involves two sites: a weak binding site and a SIM. (c) Change in structure: SUMOylation of Target C leads to changes in the conformation of the modified target due to intramolecular interactions of an internal SIM with the conjugated SUMO.

interaction surface (Fig. 3a). This has been observed, for example, in modification of the ubiquitin E2 enzyme E2-25K with SUMO, which disrupts the association between E2-25K and the ubiquitin E1 enzyme.

Formation of a novel binding site: novel complexes may be formed between a SUMOylated protein and an effector protein that contains a SUMO-interacting motif (SIM or SBM). Proteins that contain two binding sites, a SIM and a weak binding motif to protein X, will bind more strongly to this protein if it is SUMOylated (Fig. 3b). Short peptides that contain the hydrophobic core motif [V/I]-X-[V/I]-[V/I] or [V/I]-[V/I]-X-[V/I] can act as a SIM and bind to SUMO. This core is often flanked by acidic amino acids and/or serine residues.

NMR studies show that this motif forms a β strand that binds in parallel or antiparallel orientation to the β 2 strand in SUMO. One example for a SIM-mediated interaction is the association of the SIM-containing protein Daxx with sumoylated forms of GR and PML. In addition, SIMs may also play a role in target modification: First, SIMs are found in many of the enzymes involved in SUMO conjugation such as the E1 subunit Uba2, Ubc9, RanBP2, and all PIAS E3 ligases; Second, some targets, such as the transcription corepressor Daxx, need a SIM to be efficiently SUMOylated.

Changes in structure: SUMO modification can induce changes in structure if the target protein contains an internal SIM (Fig. 3c). This change was shown for the DNA excision repair enzyme thymine-DNA glycosylase (TDG): TDG binds to GU or GT mismatches and removes the mutated base. Subsequent sumoylation of DNA-bound TDG is necessary to release the enzyme from the AP site and allow for further repair. Crystallographic structural studies reveal that dissociation is due to a conformational change mediated by interaction of an internal SIM with covalently bound SUMO. Of note, conformational changes have not been observed in three sumoylated targets, RanGAP1, E2-25K, and Ets-1 that lack a SIM motif. Here, SUMO and the target are coupled in what is referred to as a “beads on a string” fashion.

Targets of SUMOylation

Based on a number of recent proteomic studies, several hundred proteins are regulated via sumoylation. They function in nearly every aspect of cell biology, including chromatin structure and function, DNA repair, signal transduction, nucleocytoplasmic transport, mitochondria fission, glucose and potassium transport, as well as basic metabolism. Transcription factors and transcriptional coregulators represent a prominent group of SUMO targets. A common feature of these proteins is that their modification leads to transcriptional repression. Interestingly, the sumoylation site in transcription factors often overlaps with an inhibitory domain known as the synergy control (SC) motif. Removal of the SC motif or conversion of the SUMO acceptor lysine to an arginine activates the transcription factor. In line with the suggestion that it is SUMO that renders these factors inactive is the finding that overexpression of SUMO isopeptidases increases the activity of transcription factors such as Sp3, the Androgen receptor (AR), and Elk1. SUMOylation does not interfere with the ability of these transcription factors to bind to DNA. Rather, the repressive function of SUMO seems to be mediated by its ability to recruit corepressors such as histone deacetylases (HDACs) to the promotor. Interestingly, HDAC1 and HDAC4 are themselves targeted for modification by SUMO. HDAC4 mutants that cannot undergo SUMOylation

display reduced transcriptional repression compared to the wild-type protein. SUMOylation also regulates the subcellular localisation of modified proteins: the cytoplasmic protein RanGAP1 is targeted to the nuclear pore complex upon modification, and PML (promyelocytic leukemia protein) sumoylation is essential for its localization to – and formation of – PML nuclear bodies. Transient recruitment of other proteins such as Sp100, Daxx, and TDG to PML bodies is dependent on their sumoylation or a SIM. Finally, SUMO has been shown to regulate enzyme and channel functions: In addition to the inhibition of E2-25K that was already mentioned above, it has recently been shown that SUMOylation inactivates the ER associated protein-tyrosine phosphatase 1B (PTP1B), alters the RNA editing properties of ADAR, and transiently inactivates potassium channels.

Competition with other Posttranslational Modifiers

Other modifications, such as ubiquitination, acetylation, or methylation, which also target lysine residues, can at least in principle compete with sumoylation. Indeed, acetylation and SUMOylation of the same lysine have been reported, for example, Mef2 or the tumor suppressor HIC1. Moreover, ubiquitin and SUMO target the same lysine residues in e.g. PCNA, I κ B α and NEMO. Phosphorylation of I κ B α leads to polyubiquitination on lysine 21, resulting in its degradation by the 26S Proteasome. SUMOylation of the same lysine residue takes place in the absence of phosphorylation. Thus, it was suggested that the role of I κ B α SUMOylation is to act as an antagonist of ubiquitin and thus protect it from proteasomal degradation. In contrast, SUMOylation of NEMO (NF- κ B essential modulator) seems to play a role in priming it for a phosphorylation-dependent ubiquitination: PIASy catalyzes the modification of NEMO by SUMO1, which in turn leads to phosphorylation by the nuclear kinase ATM. Subsequently, NEMO is deSUMOylated and ubiquitinated. This leads to translocation of NEMO to the cytoplasm, where it participates in the activation of NF κ B.

Regulation of SUMOylation

Many proteins undergo modification by SUMO only upon extra- and intracellular stimuli such as cell cycle position, nutritional state, heat shock, or DNA damage indicating that SUMOylation and/or deSUMOylation is tightly controlled. Substrate phosphorylation can act as both a positive and negative regulator of SUMOylation. Some targets such as Hsf-1, HSF4b, GATA, Mef2, and several others contain a phosphorylation-dependent SUMO modification motif (PDSM). This motif is composed of a classical SUMOylation site and an adjacent serine and proline: ψ KxE ψ SP. Heat-shock dependent phosphorylation of the PDSM in

Hsf-1 is a prerequisite for its SUMOylation. On the other hand, phosphorylation of proteins such as the transcription factors c-Jun, c-Fos, and p53 reduce their SUMOylation. While not much is known about regulation at the level of the enzymes, selected examples suggest that this may be a common mechanism. The yeast PIAS homolog Siz1 changes its localisation in mitosis from the nucleus to the bud neck, where it sumoylates septins, the mammalian E3 ligase Pc2 was reported to be regulated by phosphorylation, and H₂O₂ has been shown to reversibly inactivate SUMO enzymes. The latter is accomplished by disulfide bond formation between cysteines in the active sites of Uba2 and Ubc9. Depending on the levels of H₂O₂ this leads to global or selective deSUMOylation. A global increase in sumoylation has been observed under heat shock and osmotic stress, but the underlying mechanism is not yet clear.

Pharmacological Intervention

Since the SUMO pathway affects multiple pathways ranging from transcription, DNA repair, and intracellular trafficking over cell signaling and cell cycle control to basic metabolism, it is not surprising that links to diseases and viral assaults are emerging. However, the field is not yet at a stage sufficiently developed for pharmacological intervention. Below we will describe selected examples for links of the SUMO pathway to diseases and viral functions.

SUMO1 haploinsufficiency has been linked to a developmental defect: Based on the finding that a patient with a cleft lip and palate had a mutation in the SUMO1 gene locus, a mouse model was generated that had reduced SUMO1 expression. Increased frequency for a cleft palate or oblique facial cleft was observed in the transgenic mice, suggesting that SUMO haploinsufficiency can lead to developmental defects.

SUMO and neurodegenerative diseases: Like ubiquitin, SUMO was observed to accumulate in cytoplasmic and nuclear inclusions characteristic for diverse neurodegenerative diseases. Two proteins linked to formation of these aggregates, the natively unfolded proteins Tau and α -synuclein, are targets for sumoylation. The meaning of their modification remains however to be shown. A functional link to Huntington's disease has been suggested by the observation that a pathogenic fragment of Huntingtin (Httex1p) can be modified either by ubiquitin or by SUMO1 on identical lysine residues. Ubiquitination leads to aggregation of this fragment, whereas SUMOylation stabilizes it in a soluble state. In a drosophila model, sumoylation of Httex1p seems to exacerbate neurodegeneration.

SUMO and viruses: Different viruses have been shown to interfere with or hijack the SUMO pathway. Most drastic is the action of the CELO adenovirus

protein Gam1, which induces degradation of the SUMO E1 and E2 enzymes. Consequently, global sumoylation is lost. Other viral proteins seem more specific: Expression of the Herpes simplex virus (HSV) ICP0 protein or the Cytomegalovirus immediate early 1 (HCMV-IE1) protein leads to disruption of PML nuclear bodies and to deconjugation of SUMO from PML and Sp100. On the other hand, several immediate-early and early proteins produced by DNA viruses are themselves targets for modification or interact with components of the SUMOylation machinery.

SUMO and Cancer: While many tumor suppressors and protooncogenes can be modified by SUMO, direct links of the sumoylation pathway to cancer development are not yet available. The strongest link exists to acute promyelocytic leukemia (APL), which is caused by a chromosomal fusion between PML and the retinoic acid receptor (RARA). The resulting fusion protein PML–RARA forms homodimers that recruit and tightly bind nuclear receptors and corepressors. Transgenic mice that express a SUMO deficient mutant of the fusion protein PML–RARA do not develop APL. Other evidence is only correlative, such as the observation that SENP1 is overexpressed in human prostate cancer or that transgenic mice overexpressing SENP1 develop carcinomas at an early age.

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Superantigen

Bacterial or viral proteins linking T-cell receptors and MHC molecules through simultaneous interaction with the constant domains of all MHC class II molecules and of T-cell receptor β -chains. Hence, superantigens are polyclonal T-cell activators most likely involved in the development of autoimmune diseases.

► Interferons

Supercoils

Bacterial as well as eukaryotic chromosomes contain too much DNA to fit easily into a cell. Therefore, the DNA must be condensed (compacted) to fit into the cell or nucleus. This is accomplished by supercoiling the DNA into a highly condensed form. When relaxed circular DNA is twisted in the direction that the helix turns, the DNA becomes positively supercoiled, if it is twisted in the opposite direction, it is called negatively supercoiled. Bacterial DNA is normally found in a negatively supercoiled state. Supercoiling reactions are catalyzed by topoisomerases.

► Quinolones
► Topoisomerases

Superfamily

The term superfamily is used to include in the classical sequence-based families, proteins with no statistically significant sequence similarities but with similar three-dimensional structures.

Superoxide

Superoxide is a free radical form of oxygen (O_2^-) that is damaging to cells. Superoxide is scavenged by the enzyme superoxide dismutase used by neutrophils to destroy microbes in the body.

S

Superoxide Dismutase

Superoxide dismutase (SOD) enzymes are metalloproteins that detoxify superoxide anions (O_2^-) by converting them to H_2O_2 , which is subsequently reduced to water. SOD enzymes include the manganese (Mn) enzyme in mitochondria (SOD2) and the Cu/Zn

enzyme that is present in the cytosol (SOD1) or on extracellular surfaces (SOD3). As superoxide anions play an important role in a variety of pathophysiological conditions, such as tissue injury and inflammation, the capacity of SOD enzymes may be important for the cause of the disease. SOD mimetics are under investigation for their potential use as anti-inflammatory agents.

Suprachiasmatic Nucleus

Region of the mammalian brain residing in the anterior hypothalamus that functions as the master circadian regulator.

► Circadian Rhythms

Supraventricular Tachycardia

Supraventricular tachycardia is a heart condition characterised by fast arrhythmias involving the atrio-ventricular (AV) node.

► Antiarrhythmic Drugs

SUR

Sulfonylurea receptor (SUR), a member of the ATP-binding cassette superfamily, is a large molecular membrane protein with 17 putative transmembrane domains. The human *SUR1* gene has 39 exons and encodes the SUR1 that has high binding affinity to sulfonylureas, a class of type 2 antidiabetic drugs such as glibenclamide and glipizide, used to promote insulin secretion. The human *SUR2* gene with 38 exons encodes two types of low-affinity receptors, SUR2A and SUR2B, which are different only in the last exon.

► Antidiabetic Drugs
► K⁺ Channels
► ATP-dependent K⁺ Channel

Surrogate Ligand

Synthetic molecule or peptide that is able to activate an orphan receptor but differs in chemical structure from the natural endogenous ligand

► Orphan Receptors

Susceptibility

Susceptibility in the context of pharmacogenetics is a marginally to moderately increased risk for a disease.

► Pharmacogenetics

Swiss-Prot

Swiss-Prot is a curated databank of information on protein sequence, structure and function. It can be found under: <http://www.ebi.ac.uk/swissprot/>.

► Bioinformatics
► Molecular Modeling

SxxK Acyl Transferases

The acyl transferases of the SxxK superfamily have a specific bar code in the form of three motifs SxxK, SxN or analogue and KTG or analogue (with x denoting a variable amino acid residue). The motifs occur at equivalent places and roughly with the same spacing along the polypeptide chains, and close to each other at the immediate boundary of the catalytic centre. A constellation of bacterial genes code for SxxK acyl transferases of varying amino acid sequences, functionalities and modular designs. They occur as free-standing polypeptides and as protein fusions. Those involved in bacterial wall (4–3) peptidoglycan assembly and metabolism bind penicillin in the form of stable

and inactive penicilloyl derivatives in which the penicilloyl moiety is linked as an ester to the serine residue of the SxxK motif.

► [β-Lactam Antibiotics](#)

SxxK Free-standing Penicillin-binding Protein

SxxK free-standing penicillin-binding proteins (PBPs) are uncoupled SxxK acyl transferases that work mainly as bacterial wall peptidoglycan-hydrolases and function as auxiliary cell-cycle proteins. They are not essential.

► [β-Lactam Antibiotics](#)

SxxK β-lactamases

SxxK β-lactamases are uncoupled SxxK acyl transferases that work as β-lactam antibiotic hydrolases. They represent a mechanism of defence of great efficiency. On good β-lactam substrates, their catalytic centres can turn over 1000 times per second.

► [β-Lactam Antibiotics](#)

SxxK PBP Fusions

Class A penicillin-binding protein (PBP) fusions combine in a single polypeptide chain a class A SxxK acyl transferase linked to a glycosyl transferase module having its own five-motif bar code, itself linked to a membrane anchor. They catalyse the conversion of disaccharide-peptide units, borne by a C₅₅ undecaprenyl carrier, into polymeric (4–3) peptidoglycan. Class B PBP fusions are composed of a class B SxxK acyl transferase, a linker module having its own three-motif bar code and a membrane anchor. They are members of the morphogenetic apparatus. They are implicated in the remodelling of (4–3) peptidoglycan throughout the

bacterial cell cycle. PBP fusions of classes A and B are, globally, the lethal targets of β-lactam antibiotics.

► [β-Lactam Antibiotics](#)

Sympatholytic Drugs

Sympatholytic drugs are a group of drugs, which decrease the activity of the sympathetic nervous system, mainly by blocking the action of adrenaline and noradrenaline at adrenoceptors.

► [α-Adrenergic System](#)

► [β-Adrenergic System](#)

Sympathomimetic Drugs

Direct sympathomimetic drugs act as agonists on adrenergic receptors. In contrast, indirectly acting sympathomimetic drugs act by increasing the concentration of noradrenaline. Indirectly acting sympathomimetics like tyramine, amphetamine or ephedrine are taken up into sympathetic nerve terminals and are transported into the vesicles by the vesicular monoamine transporter in exchange for noradrenaline, which escapes into the cytosol. Most of the noradrenaline in the cytosol escapes via the transporter in the presynaptic membrane in exchange for the indirectly acting sympathomimetic monoamine. By this mechanism as well as by partly inhibiting the reuptake of noradrenaline and partly by inhibiting the degradation of noradrenaline by monoamine oxidase (MAO), they increase the concentration of noradrenaline in the synaptic cleft.

► [α-Adrenergic System](#)

► [β-Adrenergic System](#)

Synapse

A synapse is a contact site between two neurones, where information is communicated from the axon of one neurone (the presynaptic) to the cell body, the dendrites or the axon of the second neurone (the postsynaptic). In most synapses, the information is communicated chemically;

the presynaptic neurone releases a neurotransmitter substance, which then acts on receptors of the postsynaptic neurone. Most chemical synapses are characterized morphologically by an increase in diameter of the presynaptic axon to form a varicosity or bouton, by transmitter-storing vesicles inside the bouton and by a synaptic cleft crossed by cell adhesion molecules. In a relatively few synapses, the information is transmitted electrically. In a wider sense, sites of transmission between neuronal axons and peripheral effector cells, such as smooth muscle cells, are also synapses.

► Synaptic Transmission

Synaptic Plasticity

Any mechanism involved in modulating the strength of synaptic signalling (usually with higher frequency and/or amplitude of both inhibitory and excitatory neurotransmitter release). Both excitatory and inhibitory signalling can be reinforced and for both a relatively short (as in “depolarisation-induced suppression of excitation” or “inhibition”) and a relatively long (as in “long-term potentiation” and “long-term depression”) period of time.

Synaptic Transmission

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Definition

Synaptic transmission is the transfer of biological information across ►synapses. Drugs that influence synaptic transmission play an eminent role in therapy, for two reasons. First, the nervous system controls all tissues. Second, with few exceptions synaptic transmission is chemical, operating by means of transmitter substances, and synapses therefore provide a large number of drug targets, such as the enzymes that synthesize the transmitter. However, the importance of

synaptic pharmacology extends beyond therapy: many poisons act at synapses, as do the everyday drugs caffeine, ethanol and nicotine.

There are numerous transmitter substances. They include the amino acids glutamate, GABA and glycine; acetylcholine; the monoamines dopamine, noradrenaline and serotonin; the ►neuropeptides; ATP; and NO. Many neurones use not a single transmitter but two or even more, a phenomenon called ►cotransmission. Chemical synaptic transmission hence is diversified. The basic steps, however, are similar across all neurones, irrespective of their transmitter, with the exception of NO: transmitter production and vesicular storage; transmitter release; postsynaptic receptor activation; and transmitter inactivation. Figure 1 shows an overview. ►Nitrgic transmission, i.e. transmission by NO, differs from transmission by other transmitters and is not covered in this essay.

Basic Mechanisms

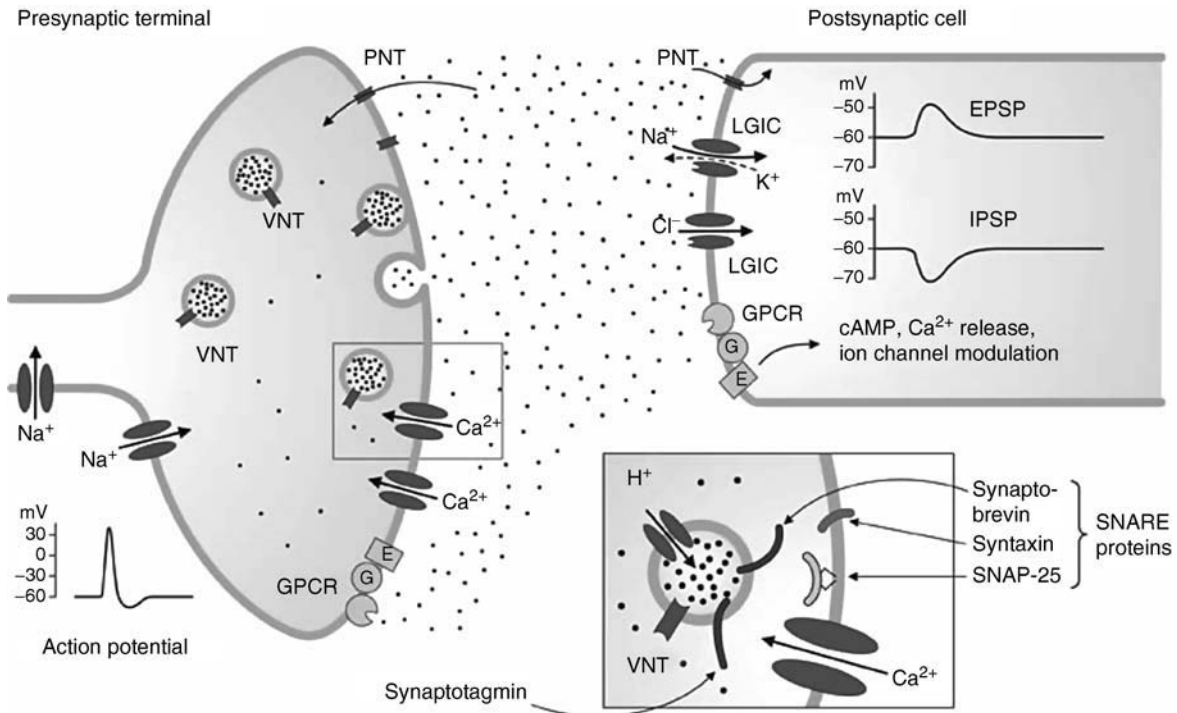
Transmitter Production and Vesicular Storage

All non-peptide transmitters are produced in the axon terminals, which possess the necessary machinery. Cholinergic terminals contain choline acetyltransferase, which acetylates choline to acetylcholine. Dopaminergic terminals contain tyrosine hydroxylase, which converts tyrosine into levodopa, and aromatic L-amino acid decarboxylase, which decarboxylates levodopa to dopamine. Noradrenergic terminals contain in addition dopamine β-hydroxylase, which oxidizes dopamine to noradrenaline. Following synthesis, the transmitters (except NO) are taken up into ►synaptic vesicles by means of a transport system consisting of two components: an ATP-driven H⁺ pump that generates a proton gradient (ΔpH; vesicle lumen acidic) and a potential gradient (Δψ; vesicle lumen positive); and the vesicular neurotransmitter transporter (VNT) (►Neurotransmitter transporters, ►Vesicular transporters), which exchanges intravesicular H⁺ with axoplasmic transmitter (Fig. 1 inset). There are several VNT families. One is the vesicular monoamine transporter (VMAT)/vesicular acetylcholine transporter (VACHT) family. VMAT is responsible for the vesicular transport of dopamine, noradrenaline and serotonin [1].

In contrast to the small transmitter molecules, the neuropeptides are synthesized in the rough endoplasmic reticulum of the neuronal perikarya. They are enclosed in vesicles in the Golgi apparatus. The vesicles travel down to the terminals by axonal transport.

Transmitter Release

All transmitters (except NO) are released by the following cascade: arrival of the nerve action potential at the terminal – opening of voltage-sensitive Ca²⁺



Synaptic Transmission. Figure 1 Synaptic transmission. The presynaptic terminal contains voltage-dependent Na^+ and Ca^{2+} channels, vesicles with a vesicular neurotransmitter transporter VNT, a plasmalemmal neurotransmitter transporter PNT, and a presynaptic G protein-coupled receptor GPCR with its G protein and its effector E; the inset also shows the vesicular H^+ pump. The postsynaptic cell contains two ligand-gated ion channels LGIC, one for Na^+ and K^+ and one for Cl^- , a postsynaptic GPCR, and a PNT. In this synapse, released transmitter is inactivated by uptake into cells.

channels – exocytosis – recycling of the vesicle membrane. The nerve action potential is mainly carried by Na^+ entry through voltage-dependent Na^+ channels. Axon terminal voltage-sensitive Ca^{2+} channels are mainly of the N- and P/Q-types.

The action potential-induced increase in axoplasmic Ca^{2+} is the physiological trigger for exocytosis. Exocytosis consists of the fusion of the vesicle membrane with the plasmalemma, the opening of a pore in the fused membranes, and outward diffusion of the neurotransmitter or neurotransmitters. Three proteins, the so-called **SNARE proteins**, are essential in exocytosis (Fig. 1 inset). One, synaptobrevin, is a protein of the vesicle membrane; the other two, syntaxin and SNAP-25 (synaptosome-associated protein of 25 kD), are proteins of the plasmalemma (Fig. 1 inset). Upon an increase in cytoplasmic Ca^{2+} from about 0.1 to about 100 μM , the vesicular synaptobrevin grabs hold of the plasmalemmal SNAREs, and the three proteins intertwine into a complex that then tightens like a zipper. This “zippering” of the SNAREs pulls the vesicle and plasma membranes together so that the lipid bilayers merge. An additional vesicular protein, synaptotagmin, may be the Ca^{2+} sensor that detects the increase in cytoplasmic Ca^{2+} . Ca^{2+}

channels and SNAREs lie closely together. Syntaxin and SNAP-25 in fact interact physically with the $\alpha 1$ subunit of N- and P/Q-type Ca^{2+} channels. The close neighbourhood permits the quickness of exocytosis; the time from the arrival of the action potential to pore formation is only 100 μs or less.

After exocytosis, the vesicle membrane with its lipids and proteins is recycled, either by immediate re-filling with transmitter or by passing through a vesicle resting pool deeper inside the axon terminal.

Action potential-elicited neurotransmitter exocytosis is variable. Firstly, action potentials trigger release from a given axon terminal only unreliably, with one or two release events per ten action potentials, resulting in a low overall release probability. Secondly, the probability of release is modulated by **presynaptic receptors** in the axon terminal membrane (Fig. 1). Through these receptors, transmitter substances from neighbouring neurones and hormones can increase or reduce the release probability [2]. Axon terminals thus integrate [1] the release command of the action potential and [2] various modulatory chemical messages from the neighbourhood to release an appropriate amount of transmitter.

Postsynaptic Receptor Activation

Of the several classes of receptors for endogenous chemical signals [3], two are used as postsynaptic receptors in synaptic transmission: ligand-gated ion channels (LGICs) and **G protein-coupled receptors** (GPCRs; Fig. 1). Due to the large number of transmitters and the existence of several receptor types for almost all, postsynaptic receptor activation is the most diversified step of synaptic transmission. Table 1 shows selected neurotransmitter receptors.

LGICs are hetero- or homo-oligomeric proteins consisting of 3, 4 or 5 peptide chains, of which each spans the membrane several times. The 3–5 subunits surround a pore, which in the absence of transmitter is closed. The nicotinic receptor for acetylcholine, the GABA_A-receptor, the glycine receptor and the 5-HT₃-receptor for serotonin are pentamers. The ionotropic glutamate receptors are tetramers. The P2X-receptors for ATP are trimers. When transmitter is bound to a ligand-gated ion channel receptor, the open probability of the pore is increased. Ions may then enter or leave the

cell, but a selectivity filter in the channel lets only certain ions pass.

The AMPA receptors for glutamate, the nicotinic acetylcholine receptor and the 5-HT₃-receptor for serotonin are cation channels (Table 1). When they open, the major consequence is a sudden entry of Na⁺, depolarization and an excitatory postsynaptic potential (EPSP; Fig. 1).

The GABA_A-receptor and the glycine receptor are Cl[−] channels (Table 1). When they open at a resting membrane potential of about −60 mV, the consequence is an entry of Cl[−], hyperpolarization and an inhibitory postsynaptic potential (IPSP; Fig. 1).

All these postsynaptic events last only for a few milliseconds; synaptic transmission through LGICs is fast. When the postsynaptic cell membrane is sufficiently depolarized, voltage-dependent Na⁺ channels open and an action potential is generated.

GPCRs are proteins that span the postsynaptic cell membrane seven times (heptahelical receptors). Small ligands are usually bound within a pocket formed by the

Synaptic Transmission. Table 1 Selected neurotransmitter receptors

Transmitter	Receptor ^a	Effector mechanisms ^b	Agonists	Antagonists
Glutamate	AMPA (LGIC)	↑ Na ⁺ and K ⁺ conductance	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)	
GABA	GABA _A (LGIC)	↑ Cl [−] conductance	Muscimol	Bicuculline
	GABA _B (GPCR)	G _{i/o}	Baclofen	
Glycine	glycine receptor (LGIC)	↑ Cl [−] conductance		Strychnine
Acetylcholine	nicotinic (LGIC)	↑ Na ⁺ , K ⁺ and Ca ²⁺ conductance	Nicotine, suxamethonium	Tubocurarine, α-conotoxins, α-bungarotoxin
	muscarinic M _{1–5} (GPCR)	M ₁ , M ₃ , M ₅ : G _{q/11} ; M ₂ , M ₄ : G _{i/o}	Muscarine, carbachol	atropine, scopolamine
Dopamine	D _{1–5} (GPCR)	D ₁ , D ₅ : G _s ; D ₂ , D ₃ , D ₄ : G _{i/o}	D ₂ : bromocriptine	D ₂ : Chlorpromazine
				D ₄ : Clozapine
Noradrenaline	α _{1A,B,D} (GPCR)	G _{q/11}	Phenylephrine	Prazosin
	α _{2A–C} (GPCR)	G _{i/o}	Clonidine	Yohimbine
	β _{1–3} (GPCR)	G _s	Isoprenaline	Propranolol
Serotonin	5-HT _{1A–F} (GPCR)	G _{i/o}	5-HT _{1B,D} : sumatriptan	
	5-HT ₃ (LGIC)	↑ Na ⁺ and K ⁺ conductance		Ondansetron
Opioid peptides	μ, δ, κ (GPCR)	G _{i/o}	μ: Morphine	Naloxone

^aLGIC, ligand-gated ion channel; GPCR, G protein-coupled receptor

^bActivation of G_{i/o} leads to ↓ cyclic AMP (inhibition of adenylyl cyclase) and ↓ Ca²⁺ conductance (N- and P/Q-type Ca²⁺ channels). Activation of G_{q/11} leads to ↑ inositol trisphosphate and ↑ diacylglycerol (stimulation of phospholipase C). Activation of G_s leads to ↑ cyclic AMP (stimulation of adenylyl cyclase)

seven transmembrane helices. The large neuropeptides bind to the extracellular domains. When the receptors are activated, they interact with the appropriate G proteins that are bound to the inner surface of the cell membrane. The G proteins then pass the information on to various effectors (Fig. 1).

The GABA_B-receptors, the muscarinic M₂- and M₄-receptors for acetylcholine, the dopamine D₂-, D₃- and D₄-receptors, the α_2 -adrenoceptors for noradrenaline, the 5-HT_{1A-F}-receptors for serotonin, and the opioid μ -, δ - and κ -receptors couple to G proteins of the G_{i/o} family and thereby lower [1] the cytoplasmic level of the second messenger cyclic AMP and [2] the open probability of N- and P/Q-type Ca²⁺ channels (Table 1). The muscarinic M₁-, M₃- and M₅-receptors for acetylcholine and the α_1 -adrenoceptors for noradrenaline couple to G proteins of the G_{q/11} family and thereby increase the cytoplasmic levels of the second messengers inositol trisphosphate and diacylglycerol (Table 1). The dopamine D₁- and D₅-receptors and the β -adrenoceptors for noradrenaline, finally, couple to G_s and thereby increase the cytoplasmic level of cyclic AMP.

These cascades of reactions need time in the range of seconds: synaptic transmission through GPCRs is slow. All further postsynaptic changes depend on the type of postsynaptic cell. For example activation of β_2 -adrenoceptors causes: in the heart an increase of the rate and force of contraction; in skeletal muscle glycogenolysis and tremor; in smooth muscle relaxation; in bronchial glands secretion; and in sympathetic nerve terminals an increase in transmitter release.

Transmitter Inactivation

Once released, transmitters are inactivated by diffusion into the neighbouring extracellular space, combined with one of two specific pathways: either extracellular degradation by enzymes that face the extracellular space, or uptake into cells.

Extracellular degradation removes acetylcholine, the neuropeptides and ATP. Acetylcholine is rapidly hydrolyzed to choline and acetate by acetylcholinesterase. The enzyme is localized in both the presynaptic and the postsynaptic cell membrane and splits about 10,000 molecules of acetylcholine per second.

Glutamate, GABA, glycine, dopamine, noradrenaline and serotonin are taken up into adjacent cells. The uptake is mediated by plasmalemmal neurotransmitter transporters (PNTs; Fig. 1). The main driving force for all PNTs is the Na⁺ concentration gradient across the axolemma. There are several PNT families. One is the Na⁺/Cl⁻-dependent neurotransmitter transporter (SCDNT) family, which comprises transporters for GABA, glycine, dopamine, noradrenaline and serotonin. The GABA transporters are located partly in the GABAergic, and the glycine transporters are located

partly in the glycinergic terminals themselves. The dopamine, noradrenaline and serotonin transporters are located almost exclusively in the dopaminergic, noradrenergic and serotonergic terminals, respectively. In all these cases, cellular uptake means re-uptake into the presynaptic neurone. Re-uptake by the PNTs may be followed by vesicular re-uptake by the VNTs; an economical way of inactivation, reminiscent of the recycling of the storage vesicle membrane [1].

Pharmacological Interventions

Transmitter Production and Vesicular Storage

Drugs affecting transmitter production and storage include the following, all acting on monoamine neurones. Levodopa is administered as the precursor of dopamine to compensate for the loss of dopaminergic neurones in ►Parkinson's disease. Benserazide is an inhibitor of aromatic L-amino acid decarboxylase that does not pass the blood–brain barrier. It is given together with levodopa in Parkinson's disease to prevent the decarboxylation of levodopa in peripheral tissues. Reserpine specifically blocks VMAT, thus depletes monoamines from the axon terminals, and is occasionally used to treat hypertension.

Transmitter Release

So central is exocytosis from neurones for animal life, that evolution has made it the target of a large number of natural poisons, used by the producing organisms for defence or attack. The puffer fish produces tetrodotoxin and some dinoflagellates produce saxitoxin, both of which occlude voltage-gated Na⁺ channels by binding to the external side of the channel pore. The cone snail *Conus geographus* produces the ►conotoxin 9-conotoxin GVIA, a small cysteine-rich peptide, which blocks N-type Ca²⁺ channels. P/Q-type Ca²⁺ channels are insensitive to 9-conotoxin GVIA but selectively blocked by the funnel-web spider venom 9-agatoxin IVA, also a small cysteine-rich peptide. Tetrodotoxin and 9-conotoxin GVIA are the two most widely used toxins in neuroscience.

The most ingenious exocytosis toxins, however, come from the anaerobic bacteria *Clostridium botulinum* and *Clostridium tetani*. The former produces the seven botulinum neurotoxins (BoNTs) A–G; the latter produces tetanus neurotoxin (TeNT). All eight toxins consist of a heavy (H) chain and a light (L) chain that are associated by an interchain S–S bond. The L-chains enter the cytosol of axon terminals. Importantly, BoNT L-chains mainly enter peripheral cholinergic terminals, whereas the TeNT L-chain mainly enters cerebral and spinal cord GABAergic and glycinergic terminals. The L-chains are the active domains of the toxins. They are zinc-endopeptidases and specifically split the three core proteins of exocytosis, i.e. the SNAREs (Fig. 1 inset). Each of the eight toxins splits a

single SNARE at a single site. Destruction of synaptobrevin, syntaxin or SNAP-25 in cholinergic terminals by the BoNTs leads to cessation of acetylcholine release followed by flaccid paralysis, the main symptom of ►**botulism**. Destruction of synaptobrevin in GABAergic and glycinergic terminals by TeNT leads to cessation of GABA and glycine release followed by spastic paralysis, the main symptom of ►**tetanus**. BoNT and TeNT are the most potent toxic substances known, able to kill vertebrates at a dose of 0.1 to 1 ng/kg body weight [4].

Postsynaptic Receptor Activation

The most diversified step of synaptic transmission is also the target of the greatest variety of drugs. Some are included in Table 1. Many are natural poisons such as muscimol, bicuculline, strychnine, nicotine, tubocurarine, muscarine, atropine, scopolamine, yohimbine and morphine, all of which stem from plants; the α -conotoxins; and α -bungarotoxin from the bungar snake. Tubocurarine, the α -conotoxins and α -bungarotoxin cause flaccid paralysis by blocking the postsynaptic nicotinic receptor of the skeletal muscle endplate – the same symptom that BoNTs produce by preventing the release of acetylcholine.

The therapeutic impact of drugs acting at transmitter receptors is enormous. All volatile and intravenous anaesthetics and also ethanol act primarily on cerebral LGICs, above all GABA_A-receptors. All neuromuscular blocking agents are agonists or antagonists at the skeletal muscle nicotinic receptor. There would be no surgery worth mentioning without these drug actions on neurotransmitter receptors. Morphine and related opioid agonists are the most effective analgesics. The β -adrenoceptor antagonists such as propranolol have become the most successful group of cardiovascular drugs. The benzodiazepine anxiolytics, which promote the effect of GABA on GABA_A-receptors, and the dopamine receptor-blocking ►**neuroleptics** such as chlorpromazine and clozapine are two major drug classes used in psychiatry.

Transmitter Inactivation

The cholinesterase inhibitors are the classical drugs that interfere with transmitter inactivation. The prototype was physostigmine, a plant poison and later a therapeutic agent in ►**myasthenia gravis**. The highly toxic nerve gases such as sarin also are cholinesterase inhibitors. The GABA transporter is blocked by tiagabine, an antiepileptic that owes its effect to the ensuing increase of the concentration of GABA in the synaptic cleft. Cocaine is abused because it blocks the dopamine transporter and, hence, enhances dopaminergic transmission in the mesolimbic dopaminergic

“reward system”. Blockers of the noradrenaline transporter and blockers of the serotonin transporter are the main ►**antidepressant drugs**, i.e. the main members of the third major drug class in psychiatry.

► α -Adrenergic System

► Catechol-O-Methyltransferase and its Inhibitors

► Exocytosis

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Synaptic Vesicle Neurotransmitter Transporters

► Vesicular Transporters

Synaptic Vesicles

Synaptic vesicles are the organelles in axon terminals that store neurotransmitters and release them by exocytosis. There are two types, the large dense-core vesicles, diameter about 90nm, that contain neuropeptides, and the small synaptic vesicles, diameter about 50nm, that contain non-peptide transmitters. About ten vesicles per synapse are “docked” to the plasma membrane and ready for release, the “readily releasable pool”. Many more vesicles per synapse are stored farther away from the plasma membrane, the “resting pool”. When needed, the latter vesicles may be recruited into the readily releasable pool. Neuronal depolarization and activation of voltage-sensitive Ca²⁺

channels increases intracellular Ca^{2+} levels that promotes exocytosis and fusion of the vesicles with the presynaptic membrane allowing the vesicular neurotransmitter to be released into the synaptic cleft.

- Exocytosis
- Synaptic Transmission
- Vesicular Transporters

Synaptotagmin

Synaptotagmin is an integral type I transmembrane glycoprotein that possesses two C2 calcium-binding domains. C2 domains are present in several other proteins such as the calcium-dependent isoforms of protein kinase C and phospholipase D where they are known to regulate Ca-dependent binding to phospholipid membranes. More than ten different synaptotagmins are known, that are widely expressed in many tissues. Best characterized is synaptotagmin I, a resident of synaptic vesicles and neurosecretory granules. Synaptotagmin I probably functions as an exocytotic Ca-receptor that links the calcium signal to membrane fusion by means of Ca-dependent binding to membranes and (possibly) to SNAREs.

- Exocytosis

Syndecans

Syndecans are transmembrane proteins, which are modified by the addition of heparan sulphate glycosaminoglycan (GAG) chains and other sugars. Syndecans bind a wide variety of different ligands via their heparan sulphate chains. Binding specificities may vary depending on cell-type specific modifications of the heparan sulphate chains.

- Table appendix: Adhesion Molecules

Synergistic Interaction

Interaction in which the combined effect is greater than the sum of the effects of each drug administered separately.

- Drug Interaction

Systems Biology

A new branch of biology using experiments and computation to gain an understanding of biological systems (e.g. a cell), taking into account complex interactions of genes, proteins, and cell elements.

- Bioinformatics

Systolic and Diastolic Blood Pressure and Pulse Pressure

Systolic pressure, or maximum blood pressure, occurs during left ventricular systole. Diastolic pressure, or minimum blood pressure, occurs during ventricular diastole. The difference between systolic and diastolic pressure is the pulse pressure. While diastolic blood pressure has been historically been used as the most relevant clinical blood pressure phenotype, it has now been clearly established that systolic blood pressure is the more important clinical predictor for cardiovascular morbidity and mortality. More recently, additional attention is focussed on the importance of pulse pressure, i.e. the blood pressure amplitude, as a predictive factor for cardiovascular disease.

- Blood Pressure Control

t_{1/2} or t_{1/2β}

- Half-life / Elimination Half-life
- Pharmacokinetics

T₃

- Triiodothyronine

T₄

- Thyroxine

T Cell Anergy

Mechanism of peripheral immune tolerance characterized by a state of functional unresponsiveness induced in T cells by suboptimal or partial stimulation. Engagement of the antigen receptor in the absence of costimulation signaling induces anergy in T cells, which become unable to respond to new stimulations.

- NFAT Family of Transcription Factors
- Immune System

T Cell Antigen Receptor

- T Cell Receptors

T Cell Receptors

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Synonyms

T cell antigen receptor; T cell receptor; TCR

Definition

The T cell receptor is a clonotypic, T cell specific surface receptor that mostly recognizes peptides bound to MHC molecules on antigen-presenting cells.

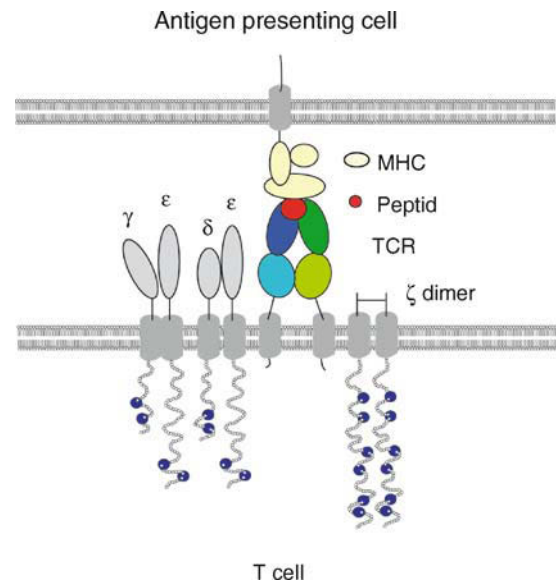
Basic Characteristics

The immune system of vertebrates has witnessed the evolution of an adaptive arm of the immune response that allows for the specific and persistent response to pathogens. At the heart of this development lies the ability of B and T cells to produce clonotypic receptors (referred to as BCR and TCR from hereon) that constantly monitor the fluidal and cellular composition of the body and potentially elicit an immune response should foreign or danger signals exceed a certain threshold value. Antigen recognition by the heterodimeric BCR and TCR is achieved by loop regions – also called complementarity determining regions (►CDR's) – of their respective variable domains. However, while the BCR, antibodies and a small subset of TCR's called β-δ-TCR's directly recognize diverse structural features including proteins, RNA, DNA or lipids, the α-β TCR has evolved almost exclusively towards the recognition of peptides derived from proteolytically cleaved proteins. Consequently, while BCRs, antibodies mostly recognize antigens in their native molecular context and conformation, TCRs interact with immunogenic peptides embedded in the binding groove of the body's own major histocompatibility complex (MHC) molecules. Seminal experiments by Zinkernagel and Doherty in the 1970's, where they analyzed cytolytic T cell responses of mice infected by lymphocytic choriomeningitis virus, introduced this concept of "MHC restriction" [1]. Without detailed knowledge of the structure and composition of the

molecules involved, they hypothesized that a single receptor is responsible for the recognition of a virus-induced modification of the self-MHC. This “altered-self” hypothesis was later proven more directly with the advent of molecular biology and the cloning of individual TCRs [2]. However, the bold interpretation of their intriguing findings won Zinkernagel and Doherty the Nobel Prize in 1996.

Another central aspect of B and T cell immunology has long been the question as to how variability at the level of individual cells and individual molecules could be achieved. The clonal selection theory by McFarlane Burnet set the stage for deciphering the recombination events that govern immunoglobulin and TCR gene rearrangements [3]. Combinatorial recombination events of families of gene segments leads to a very diverse set of B and T cell receptors in somatic cells that are constantly selected on the basis of BCR or TCR activation thresholds matched to distinguish self from non-self. The precursors of T cells, the common lymphoid progenitor cells, are produced in the bone marrow, and they are committed to T cell fate after transport to the ▶thymus where they further mature. During thymic development, β chain rearrangements are followed by recombination of the α chains of the TCR. Productive rearrangements are required for thymocyte survival and TCR signaling seems to be pivotal for development. On the one hand, the thymocytes with productively rearranged TCRs need to recognize self-MHC to become “MHC restricted,” while on the other hand the binding to MHC in complex with self-peptide should not lead to a strong response, since this may pose problems after the release of such T cells into the periphery (potentially leading to strong anti-self reactions and autoimmunity).

While the T cell receptor α/β -heterodimer is the central element of receptor adaptivity, there are additional components that non-covalently associate and directly contribute to a functional TCR [4] (Fig. 1). All of these additional components, namely CD3 ϵ , CD3 γ , CD3 δ and the ζ dimer, have cytoplasmic tails that contain so-called immune receptor-tyrosine based activation motifs (▶ITAMs). The ITAMs can become tyrosine phosphorylated upon stimulation, and thereby they act as docking sites for the SH2 domains of the ZAP-70 kinase. Signaling cascades are subsequently initiated that lead to Ca^{2+} mobilization, changes in integrin-mediated cell adhesion, and the transcription of genes [5]. Extracellular binding of components of the CD3 complex, for the purpose of modulating T cell receptor mediated responses, are not only potentially modulating the interaction of the receptor with the MHC:peptide complex. More importantly they elicit or attenuate signaling events mediated by conformational changes or clustering of the receptors and induce the



T Cell Receptors. Figure 1 Composition of the TCR/CD3 complex. The extracellular domains of the α/β heterodimer are shown in blue and green and the counteracting peptide:MHC is shown in red and yellow, respectively. In this example, the peptide binding groove is represented by a single chain, which is indicative of MHC class I molecules. The additional components of the TCR/CD3 complex, which are critical for intracellular signaling, are depicted as grey ovals, cylinders represent transmembrane domains and the dotted structures stand for the cytoplasmic domains of the complex. Dark blue dots highlight the tyrosine residues of the ITAM motifs that can become phosphorylated upon receptor stimulation.

concomitant post-translational modifications of the cytosolic parts of the CD3 complex.

Drugs

CD3 Specific Monoclonal Antibodies

Introduction/Overview

The first mouse ▶monoclonal antibody specific for human CD3 was produced in 1979 and named “orthoclone OKT3.” Aside from its use in the laboratory, OKT3 became the first anti-CD3 antibody to be utilized in transplantation medicine, but its wider application was hampered by its immunogenic and mitogenic properties (reviewed in [6]). Consequently, humanized and engineered anti-CD3 antibodies were developed to circumvent these limitations (Table 1). Since T cells and the TCR are involved in many immunological diseases, it is not surprising that the application of CD3 antibodies is not restricted to the field of transplantation. For example, CD3 antibodies are tested in clinical studies of diseases such as autoimmune diabetes (type 1 diabetes), immune-mediated inflammatory arthritis and inflammatory bowel disease [7].

Side Effects – Immunogenicity and Mitogenicity

In the early phase of application a massive humoral immune response was raised by CD3 antibodies which reduced their potential clinical benefit. Furthermore, 5–7 days after administration of OKT3 CD3 antibodies, OKT3 was neutralized and cleared by the recipient's immune system. Initially, attempts were made to suppress the antibody-induced immune response by co-administration of conventional immunosuppressive drugs like corticosteroids or azathioprine. More recently attempts have been made to reduce the immunogenicity of the CD3 antibodies themselves. “Humanized” CD3 antibodies (Fig. 2) contain the hypervariable region of the parental rodent antibody which are grafted onto a human heavy- and light-chain immunoglobulin. While humanization is intended to attenuate the number of potentially immunogenic **epitopes**, mutations that abolish **Fc receptor** binding are able to reduce the negative side effects of the antibody's effector function. In a more radical approach, a fully human framework might be used for selection of the desired anti-CD3

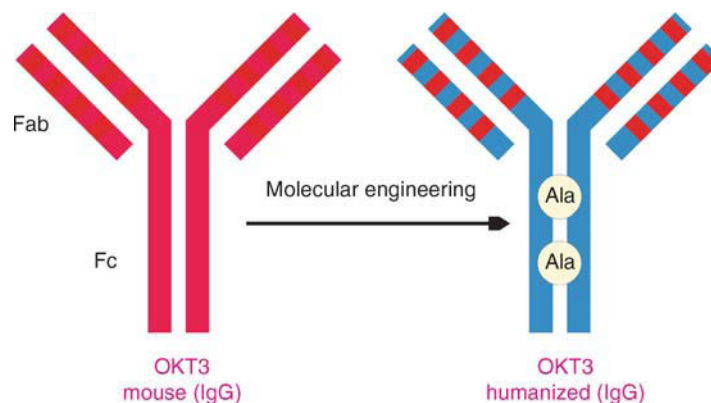
binding specificity, e.g. by phage display, and the corresponding Fab fragments may then be either used directly or combined with a non-mitogenic Fc part of an antibody. In contrast to these modern versions of CD3 antibodies, early CD3 antibodies were mitogenic *in vitro*, inducing T cell proliferation and cytokine production, features typical for activated T cells and an activated immune system in general. Surprisingly, the same antibodies *in vivo* induced immunosuppression which led to the tolerance of transplanted organs. This apparent paradox was the starting point of several interesting studies which sought to shed light on the molecular mechanisms underlying the mode of action of CD3 antibodies [6].

Mode of Action

The current models for the mode of action of T-cell-specific monoclonal antibodies are based on the observation that the administration of CD3 antibodies leads to a significant reduction of the number of CD3-positive T cells. On the one hand, CD3⁺ T cells were

T Cell Receptors. Table 1 Important hallmarks in the development of anti-CD3 antibodies [6,7,12]

Therapeutic CD3 antibodies – Stages of development	
1979	First CD3-specific monoclonal antibody: OKT3
1981	First clinical trials with OKT3 (therapy of renal allograft rejection)
1985	Commercial availability of OKT3
1993/1994	First humanized non-Fc receptor (FcR)-binding CD3-antibodies
1999	Proof of safety for non-FcR-binding CD3-antibodies (phase I clinical trials)
2002	First trials with CD3 antibodies for therapy of autoimmune diseases (insulin-dependent diabetes mellitus; psoriatic arthritis)
2005/2007	Trials for therapy of inflammatory bowel diseases



T Cell Receptors. Figure 2 Humanized CD3 antibodies. Starting from mouse monoclonal CD3-specific OKT3 antibodies, so called “humanized antibodies” are designed and produced using recombinant techniques. Mouse hypervariable regions (displayed in red) within the Fab part of the antibody are transferred from the mouse antibody onto a human framework. Certain mutations can be introduced within the Fc region (e.g. the double mutant Leu234Ala/Leu235Ala in OKT3), which act to destroy the antibody's Fc-receptor binding capacity and potentially reduce mitogenicity.

depleted by sustained stimulation and led to activation induced cell death. On the other hand, a substantial number of cells remained physically present but displayed reduced CD3/TCR surface expression while other T cell molecules like CD4 or CD8 were left unaffected (Fig. 3a). These CD3/TCR-negative cells are not susceptible to antigen-specific signals and thereby contribute to an increased immunotolerance. CD3 antibody induced TCR downmodulation is fully reversible. After clearing CD3 antibodies from the blood, expression of the CD3/TCR complexes is fully restored within a few hours [6].

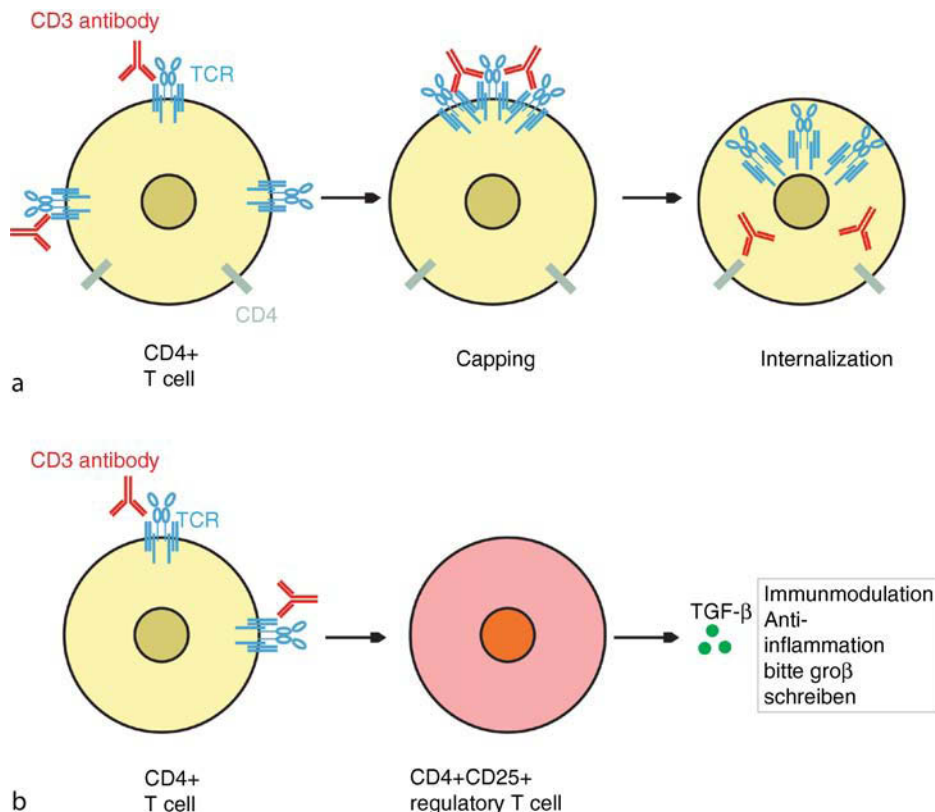
In a mouse model for autoimmune non-obese diabetes (NOD), the full-blown diabetes could be reversed to normoglycaemia by treatment with CD3 antibodies. In these diabetes-tolerant mice, the proportions of CD4⁺ CD25⁺ regulatory T cells were increased. Moreover, the CD4⁺ CD25⁺ regulatory T cells of the tolerant mice produced high levels of

TGF- β . Neutralizing antibodies specific for TGF- β prevented the CD3-specific antibody-induced diabetes remission. These observations led to the hypothesis that CD3 antibodies are acting through cell signaling events, including the generation of CD4⁺ CD25⁺ regulatory T cells and the secretion of TGF- β (Fig. 3b). It will be interesting to see whether the correlation between CD3 antibody administration and the number of regulatory T cells will hold for other disease models as well.

Alternative Approaches to CD3 Antibodies

TCR α/β Antibodies

In addition to antibodies targeting the CD3 subunit of the TCR complex, antibodies against the α and β subunits of the TCR have been tested as therapeutic agents. A benefit for the treatment experimental autoimmune encephalomyelitis or collagen induced arthritis could be shown in animal models [8].



T Cell Receptors. Figure 3 Schematic view of the putative mode of action of anti-CD3 antibodies. A, TCR downmodulation. In hosts treated with CD3-specific antibodies special subpopulations of T cells can be detected. These T cells are negative for their CD3/TCR but positive for other T cell markers like CD4. The crosslinkage of several CD3/TCR complexes via the CD3 antibodies leads to a local enrichment of CD3/TCRs complexes (capping). The CD3/TCR complexes are subsequently internalized and either recycled or degraded. B, Antibody-induced production of TGF- β mediated by regulatory T cells. This scheme is based on mouse models that show the administration of CD3 antibodies to correlate with increased number of regulatory T cells and the reduction of autoimmune symptoms. This effect could be reversed by the reduction of TGF- β via administration of TGF- β -neutralizing antibodies.

Soluble T Cell Receptors

In addition to the antibodies described above, latest research considers the TCR α/β heterodimer itself as a promising protein for immunotherapies. Considering the diversity of naturally occurring T cell receptors they might represent interesting scaffolds for the generation of novel, highly specific therapeutics. Utilizing the methods of directed evolution (e.g. DNA shuffling and phage display), artificially modified, recombinant and soluble TCRs can be produced. Thereby, the issues of stability, dissociation rates from the MHC:peptide complex, and peptide specificity can be tackled. Soluble TCRs might also represent interesting therapeutics for cancer immunology. Like therapeutic antibodies, soluble TCRs could be engineered to precisely deliver radionuclids, toxins or immunomodulatory molecules to their intended site of action. Moreover, the application of soluble TCRs for the treatment of autoimmunity (e.g. for targeted delivery of immune-inhibitory lymphokines) or as agents for vaccination in the generation of autoimmunity-suppressing regulatory T cells is possible and first experimental studies have been undertaken [9,10].

Outlook and Risks

The immune system plays a fundamental role in maintaining the physiologic integrity of the human body, and pharmacological immunomodulation is therefore a field of continuous development. With the TCR/CD3 complex as a central player determining the specificity of the immune response, this complex constitutes a prime target for intervention. As an understanding of the general principles governing T cell receptor signaling is being unraveled, it is now time to exploit these features in a more sophisticated manner by addressing various diseases. Recent clinical trials have identified the therapeutic potential of CD3 antibodies and their more advanced derivatives. In this context, CD3 antibodies were tested for treatment of psoriatic arthritis, acute renal allograft rejection, pancreatic islet allograft rejection, Crohn's disease and severe ulcerative colitis. Since immunotherapy always imposes a potential risk to the intricate homeostasis of the patients immune system itself, the success of these approaches is still closely related to an advanced understanding of the underlying molecular mechanisms involved, and will require ongoing basic and preclinical research [11].

► NFAT Family of Transcription Factors

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T-cells

► T-lymphocytes

Tachykinins and their Receptors

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Synonyms

Neurokinins (Table 1); Neurokinin receptors (Table 1)

Definition

Except substance P (SP), the first mammalian tachykinin (TK) peptide to be sequenced (by Chang and Leeman in 1970), other peptides belonging to TK family have synonyms. This is due to historical reasons,

in 1983 three different groups (Kimura, Matsuo, Nawa and their respective coworkers) independently identified other TK peptides: these peptides were renamed as neurokinin A (NKA) and neurokinin B (NKB) at the Montreal Symposium in 1986 when a new nomenclature system was proposed (Table 1). Unfortunately, since no general agreement on this nomenclature was reached, these synonyms have been also the basis to define the receptors at which each of the above TK acts as preferential agonist.

Tachykinin Peptides

TKs are a family of peptides widely expressed in the central nervous system and peripheral tissues of vertebrates and invertebrates [1, 5]. All TKs share a common C-terminal amidated amino acid region, Phe-X-Gly-Leu-MetNH₂, which is the minimal sequence for exerting biological activity at TK receptors (Table 2). SP, NKA and its elongated forms, and NKB have conserved amino acid sequences across the mammalian species investigated so far, whereas the rodent (rat and mouse) sequence of hemokinin-1 (HK-1) differs from that found in humans, although the pharmacological profile of rodent and human HK-1 are very similar [1, 4, 5].

Genes for Tachykinin Peptides

All TKs are encoded by three genes called *TAC1*, *TAC3*, and *TAC4* (see Table 1 for synonyms). *TAC* genes are transcribed into primary mRNAs which are then

subjected to alternative posttranscriptional splicing (Table 3). The translation of alternatively spliced mRNAs by ribosomes located on the surface of the rough endoplasmic reticulum produces pre-pro-TK peptides which are then subjected to posttranslational processing to generate pro-TK peptides, through the removal of the signal sequence. Finally, following the removal of spacer sequences of the pro-peptides and the C-terminal amidation of methionine, mature TKs are now ready to be released into the extracellular environment where they exert their biological actions through the stimulation of specific receptors located within the plasma membrane.

Tachykinin Receptors

TKs exert their biological effects through three distinct receptor (r) types termed TK NK₁r, NK₂r, and NK₃r (see Table 1 for synonyms). These receptors belong to the class 1 (rhodopsin-like), seven transmembrane, G-protein-coupled receptors (capability to bind and hydrolyse GTP). Since TK receptors are proteins (i.e., large peptides), the scheme of their biosynthesis is similar to that of TKs (Table 3). The amino acid sequence of TK receptors shows slight species-related variations which account for the species-related differences in the affinity of some receptor antagonists; in contrast, critical residues for the binding and intrinsic activity of TKs are phylogenetically conserved [1, 5].

Tachykinins and their Receptors. Table 1 Abbreviations and synonyms of tachykinin peptides, genes, and receptors

Main Term (Montreal nomenclature and extensions)	Abbreviations	Synonyms
Tachykinin	TK	Neurokinin
Substance P	SP	—
Neurokinin A	NKA	Substance K, Neurokinin-alpha, Neuromedin L
Neurokinin B	NKB	Neurokinin-beta, Neuromedin K
Hemokinin-1	HK-1	—
Endokinin A	EKA	—
Endokinin B	EKB	—
Tachykinin NK ₁ receptor	TK NK ₁ r	Neurokinin-1 receptor, substance P receptor
Tachykinin NK ₂ receptor	TK NK ₂ r	Neurokinin-2 receptor, neurokinin A receptor, substance K receptor, neurokinin-alpha receptor, Neuromedin L receptor
Tachykinin NK ₃ receptor	TK NK ₃ r	Neurokinin-3 receptor, neurokinin B receptor, neurokinin-beta receptor, Neuromedin K receptor
Tachykinin 1 gene	<i>TAC1</i> , <i>PPT-A</i> , <i>PPT-I</i>	Preprotachykinin A gene, Preprotachykinin I gene
Tachykinin 3 gene	<i>TAC3</i> , <i>PPT-B</i> , <i>PPT-II</i>	Preprotachykinin B gene, Preprotachykinin II gene
Tachykinin 4 gene	<i>TAC4</i> , <i>PPT-C</i>	Preprotachykinin C gene,
Tachykinin NK ₁ receptor gene	<i>TACR1</i>	—
Tachykinin NK ₂ receptor gene	<i>TACR2</i>	—
Tachykinin NK ₃ receptor gene	<i>TACR3</i>	—

Tachykinins and their Receptors. Table 2 Peptide sequences of mammalian tachykinins

Substance P	RPKPQQFF GLM -NH ₂
Neurokinin A	HKTDSFVGLM -NH ₂
Neurokinin A (3–10)	TDSFVGLM -NH ₂
Neuropeptide-gamma	DAGHGQISHKR HKTDSFVGLM -NH ₂
Neuropeptide-kappa	DADSSIEKQVALLKALYGHGQISHKR HKTDSFVGLM -NH ₂
Neurokinin B	DMHDF FVGLM -NH ₂
Hemokinin-1	TGKASQFFGLM -NH ₂
Endokinin A	DGGEETQLSTEAEETWVIVALEEGAGPSIQLQLQEVK TGKASQFFGLM -NH ₂
Endokinin B	DGGEETQLSTEAEETWEGAGPSIQLQLQEVK TGKASQFFGLM -NH ₂
Endokinin C	KKAYQLEHT FQGLL -NH ₂
Endokinin D	VGAYQLEHT FQGLL -NH ₂
Rat and mouse Hemokinin-1	RSRTRQ FYGLM -NH ₂

The common C-terminal amino acid sequence required for exerting activity at tachykinin receptors is shown in bold: endokinin C and D lack the C-terminal Met and are almost devoid of affinity at these receptors. In red, the sequence of neurokinin A of which neuropeptide-gamma and neuropeptide-kappa are elongated forms and neurokinin A (3–10) is a product of *beta* or *gamma*-TAC1 mRNAs or an NKA metabolite active at tachykinin receptors. In blue, the sequence of human HK-1 of which endokinin A and B are elongated forms.

Tachykinins and their Receptors. Table 3 Human TK and TK receptor genes (DNA), TK and TK receptor-encoding mRNAs, and TK peptides and TK receptor proteins

Gene (DNA)	mRNA	Peptides or proteins
TAC1	alpha-TAC1	Substance P
	beta-TAC1	Substance P, neurokinin A, neurokinin A (3–10), neuropeptide-K
	gamma-TAC1	Substance P, neurokinin A, neurokinin A (3–10), neuropeptide-gamma
	delta-TAC1	Substance P
TAC3	alpha-TAC3	Neurokinin B
	beta-TAC3	Neurokinin B
	gamma-TAC3	None
TAC4	alpha-TAC4	Hemokinin-1, hemokinin-1 (4–11), endokinin A, endokinin B, endokinin C
	beta-TAC4	Hemokinin-1, hemokinin-1 (4–11), endokinin B, endokinin D
	gamma-TAC4	Hemokinin-1, hemokinin-1 (4–11), endokinin B
	delta-TAC4	Hemokinin-1, hemokinin-1 (4–11), endokinin B
TACR1	long-TACR1	Long NK ₁ receptor (407 amino acids)
	short-TACR1 (encode for a shorter, 7 amino acids C-terminal intracellular sequence)	Short NK ₁ receptor (311 amino acids)
TACR2	alpha-TACR2 (classic)	Alpha-NK ₂ receptor (398 amino acids)
	Beta-TACR2 (encode for a shorter receptor lacking extracellular (EC3), and intracellular (IC2) loops and a transmembrane domain (TM4))	Beta-NK ₂ receptor (333 amino acids)
TACR3	TACR3 (classic)	NK ₃ receptor (465 amino acids)
	TACR3 variant (encode for a shorter receptor putatively of lacking extracellular (EC2), and intracellular (IC2) loops and a transmembrane domain (TM4))	Protein expression not yet demonstrated

Genes for Tachykinin Receptors

TK NK₁r, NK₂r, and NK₃r are encoded by three genes termed as *TACR1*, *TACR2*, and *TACR3*, respectively. Splice variants of these TK receptors have also been identified (Table 3).

Basic Characteristics

The binding of TKs to their receptors promotes conformational changes enabling the receptors to couple with specific ▶heterotrimeric GTP-binding proteins (made up by alpha, beta, and gamma subunits) which in turn activate enzymes capable of generating second messengers; this process is defined as signal transduction. These second messengers diffuse in the intracellular environment and trigger a cascade of events leading ultimately to the cellular responses [3]. These events include the generation of other mediators like prostanoids and nitric oxide which can diffuse through the plasma membrane, or the extracellular release of other neurotransmitters (i.e., acetylcholine, noradrenaline, dopamine, GABA, glutamic acid) thus affecting the functionality of neighbor cells [5]. Albeit with different relative potency, SP, NKA, NKB, HK-1, elongated forms of NKA (neuropeptide gamma and kappa) and HK-1 (endokinin A and B) act as agonists at NK₁ (NK₁r), NK₂ (NK₂r), and NK₃ receptors (NK₃r), whereas endokinin C and D are virtually inactive at these receptors, or, in some systems, they act as NK₁r antagonists. This also applies to TK metabolites, where NKA(3–10) (that can be also directly translated from *beta-TAC1* and *gamma-TAC1*) is a good agonist at TK receptors, whereas the N-terminal metabolite SP(1–7) does not interact with TK receptors, although it exerts several biological activities through a yet unidentified molecular target.

The structure of all TK receptors is similar in terms of expression of *TACR* genes, since all these genes contain five exons intercalated by four introns [1, 5]. Exon I encodes for the N-terminal extracellular tail, the first intracellular (IC1) and extracellular (EC1) loops and the first, second, and third transmembrane domains (TM1, TM2, and TM3). Exon II encodes for the second intracellular (IC2) and extracellular (EC2) loops and the fourth transmembrane domain (TM4). Exon III encodes for the fifth transmembrane domain (TM5) and the third intracellular loop (IC3). Exon IV encodes for the sixth and seventh transmembrane domains (TM6 and TM7) and the third extracellular loop. Exon V encodes for the C-terminal intracellular tail only. A schematic drawing of the amino acid sequences and TK receptor organization is shown in Fig. 1.

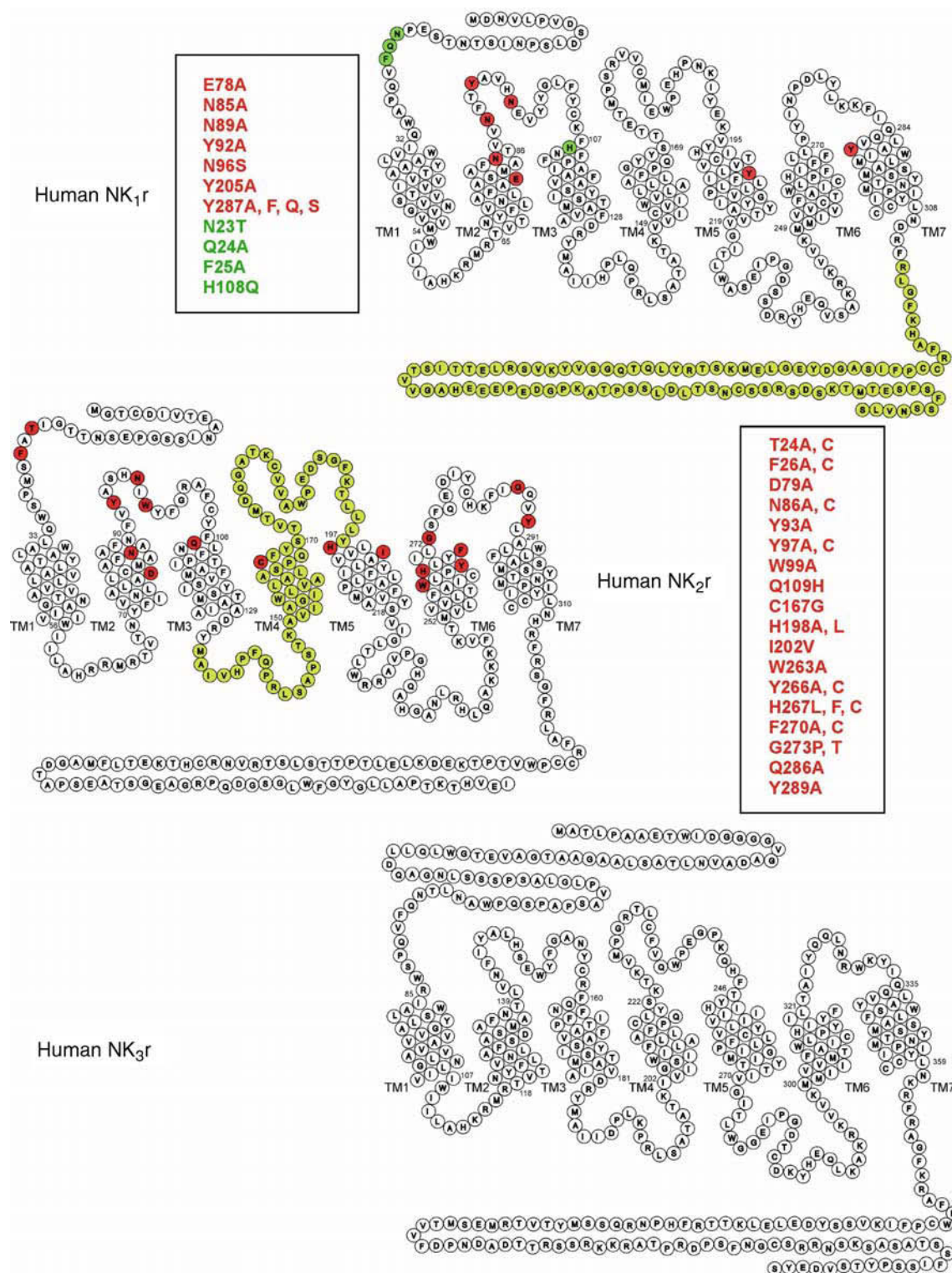
Available evidence indicates that the stimulation of NK₁r, NK₂r, or NK₃r by the respective preferred endogenous agonist, that is, SP, NKA, and NKB respectively, lead to similar basic transduction mechanisms mediated by the coupling with G-proteins such as Galpha-q and Galpha-s [1, 3, 5]. As a consequence,

the respective activation of NK₁r, NK₂r, or NK₃r by SP, NKA, and NKB increases the activity of phospholipase C via Galpha-q and thus promote the hydrolysis of the phospholipid phosphatidyl inositol triphosphate into diacylglycerol and inositol triphosphate which increases intracellular calcium by releasing it from endoplasmic reticulum. Likewise, NK₁r, NK₂r, or NK₃r-mediated activation of Galpha-s stimulates the activity of adenylate cyclase therefore increasing intracellular levels of cAMP. These two transduction pathways can either coexist or be specific for a given cell system. Because of these common transduction pathways, the selective stimulation of either NK₁r or NK₂r can evoke similar functional responses (e.g., contraction) in cell systems which can express both receptors (e.g., smooth muscle cells). On the other hand, the effects of the stimulation of different TK receptors can diverge when looking at other cell systems or other cellular responses; for instance, the stimulation of NK₁r induces the proliferation of hematopoietic stem cells, whereas the stimulation of NK₂r inhibits their proliferation. This would imply that along transduction pathways common to all TK receptors there should be other ones that are receptor specific. Moreover, it should be pointed out, that different TKs acting at a single TK receptor have a different probability to induce receptor conformers responsible for a different G-protein coupling [3]. As a consequence, different TK agonists acting through the same TK receptor can generate different cellular or tissue responses. Furthermore, the same agonist can induce different responses by activating the same TK receptor on different cells, since the qualitative or quantitative expression of enzymes transducing the receptor signal can vary from cell type or even within different clones of the same cellular type. Overall, several examples of TK ▶co-transmission have been described in the physiology of both laboratory animals and humans. The vast majority of *TAC1*-expressing neurons (CNS, enteric, sensory) corelease multiple TKs having preferential affinity for NK₁r and NK₂r. These receptors are often coexpressed on target cells (e.g., smooth muscle, secretory elements) and the contribution of the single receptor to the overall response mediated by the release of endogenous TKs can vary case by case (Table 4).

Basic agonist pharmacology at NK₁r, NK₂r, or NK₃r that is valid for most of bioassay is shown in Table 5.

Tachykinin NK₁ Receptors

Two NK₁r splice variants have been identified (Table 3). A NK₁r splice variant having a very short C-terminal intracellular tail (7 instead of 96 amino acids), which has been expressed and characterized in recombinant systems (Fig. 1), was found to be expressed at higher level than the long isoform in breast cancer cells. As compared to the long receptor, the short NK₁r isoform is less subjected to desensitization and internalization



Tachykinins and their Receptors. Figure 1 Snake plots of human tachykinin NK₁, NK₂, and NK₃ receptors. Amino acid sequences and receptor organisation have been downloaded from www.gpcr.org/7tm/seq/. In yellow are indicated amino acids which lack the short NK₁ and beta-NK₂ receptor isoforms. Amino acids whose substitution can lead to a significant decrease (>10-fold) in SP and NKA binding affinity at NK₁ and NK₂ receptors, respectively, as detected through homologous competition are marked in pink and those detected through heterologous competition (i.e., displacement of the NK₁r antagonist [¹²⁵I]-703606) are marked in green in both the snake plots and in the respective insert within the figure. (Courtesy of Dr. Stefania Meini.)

Tachykinins and their Receptors. Table 4 Examples of tachykinin cotransmission

Type of cotransmission	Examples
Summation	The overall response is the arithmetic sum of the contribution brought by each receptor (e.g., NK ₁ and NK ₂). Capsaicin-induced contraction of rat isolated urinary bladder, electrical field stimulation (EFS)-induced nonadrenergic, noncholinergic contraction of the isolated guinea pig bronchus or renal pelvis when SP metabolism is blocked by peptidase inhibitors are all examples of summation. Both NK ₁ r and NK ₂ r antagonists are required to abolish such kind of response, but each antagonist substantially reduces the response
Cooperation	The overall response is qualitatively different from the response mediated by each receptor. EFS-induced nonadrenergic, noncholinergic contraction in guinea pig or human small intestine circular muscle is associated to the generation of muscle action potentials. Both responses can be blocked by either NK ₁ r or NK ₂ r antagonists indicating that the costimulation of both receptors is required for eliciting these responses
Specialization	Each receptor contributes to morphologically (e.g., phasic vs. tonic) or temporally (early vs. late) distinct components of the overall response. This is the case of prolonged EFS-induced nonadrenergic, noncholinergic contraction the guinea pig colon or rat urinary bladder, where the fast and late components of the contraction are mediated by NK ₁ r or NK ₂ r, respectively
Supra-additivity	This is a special case of cooperation where the blockade of a single receptor is not sufficient to reduce the overall response. Capsaicin-induced nonadrenergic, noncholinergic contraction in the guinea pig ileum or common bile duct can be inhibited only when at least two different TK receptors are blocked (e.g., NK ₁ + NK ₂ , NK ₁ + NK ₃ , NK ₂ + NK ₃)

Tachykinins and their Receptors. Table 5 Order of potency of endogenous human TKs at NK₁, NK₂, and NK₃ receptors and selective TK NK₁, NK₂, and NK₃ receptor agonists

Receptor	Natural agonists (order of potency)	Selective agonists
NK ₁	SP = HK-1 > NKA > NKB	SP-methylesther, [Pro ⁹]-SP, [Sar ⁹ , Met(0 ₂) ¹¹]-SP, septide, GR73632
NK ₂	NKA > NKB > SP = HK-1	[βAla ⁸]-NKA(4–10), [Nle ¹⁰]-NKA(4–10), [Lys ⁵ , MeLeu ⁹ , Nle ¹⁰]-NKA(4–10), GR64349
NK ₃	NKB > NKA > HK-1 ≥ SP	[MePhe ⁷]-NKB, [MePhe ⁷]-NKB(4–10), senktide

upon stimulation with SP [3], and thus it produces a more effective stimulus for the autocrine induction of *beta-TAC1* expression and proliferation of these cells. Despite these different characteristics, no major pharmacological differences for the 2 NK₁r splice variants have been reported yet.

Receptor residues critical for the modulation of SP binding have been identified in the extracellular domains, and in particular in the N-terminal tail, EC1 and EC2 loops, and in the outer surface of TM2 and TM7 (see Fig. 1 for critical point mutations) [1]. Although, among natural TKs, SP is the more potent agonist at NK₁r in all systems known so far (or at least equipotent to HK-1), NKA is also a very good agonist when these receptors are coupled to Galph_q, and there is evidence that NKA is an endogenous NK₁r agonist in some districts (eye, spinal cord) [3]. All NK₁r agonists are relatively less potent to induce cAMP accumulation than inositol phosphates formation [3, 5]. Indeed, NKA and some selective NK₁ agonists, such as septide, are

partial agonists at these receptors when they are coupled to Galph_s; this could be the reason why septide is less effective than other NK₁r agonists in the stimulation of nitric oxide production. G166 and Y167 residues, located on the upper edge of the TM4 domain, play a key role in SP-induced coupling of NK₁r to Galph_s [1, 5].

At the beginning of 1990s, a discrepancy between the apparent affinity of septide (and other NK₁r agonists) measured through cellular responses or SP displacement emerged: septide potently (nanomolar range) activated NK₁r-mediated cellular signaling (inositol phosphate production, calcium response), whereas it was a weak displacer of SP binding (micromolar range). Furthermore, the apparent affinity of NK₁r antagonists was higher when measured against the responses induced by septide than those induced by SP (or some other NK₁r agonists). These results led to the definition of a septide-sensitive NK₁r. Although the intimate mechanisms underlying these discrepancies have been not completely clarified, it is now assessed that the

behavior of septide-sensitive receptor is not due to different genotypes or phenotypes (intended as amino acid sequence) of NK₁r, but rather to a different dynamic of agonist–receptor interactions within a single transduction pathway [1, 3].

NK₁r also couple with Go, which increases the activity of phospholipase A2 leading to the formation of arachidonic acid and then prostanoids. SP via NK₁r is also known to affect the activity of several ion channels. Although these effects could involve effectors located downstream to the above mentioned transduction pathways, such as protein kinase C (PKC) activation, recent evidence indicates the inhibition of inward rectifier potassium channel by SP would involve a direct interaction of Galpha-q with the channel. A further mechanism through which TKs acting at NK₁r modulate channel activity involves beta/gamma subunits of G-proteins which dissociate from Galpha subunit upon receptor stimulation. This mechanism is responsible for the fast inhibition of R-type calcium channels by NKA (via NK₁r) in a recombinant system.

Agonist-triggered events in receptor dynamics have been termed as **receptor trafficking**: these events are intimately related to the agonist-induced cellular responses [2]. Beta-arrestins play an important role in tachykinin receptor trafficking (uncoupling, internalization, MAPK activation) but, downstream to beta-arrestins, other proteins are also involved in receptor trafficking, as shown in recombinant or native systems expressing NK₁ receptors [2]. The GTPase dynamin is involved in the formation of early endosomes and resensitization of SP response (calcium mobilization) following an initial SP challenge causing desensitization. Dynamin also plays an essential role in SP-induced MAPK activation. Ras-related 5a (Rab5a) is another GTPase involved in the formation of clathrin-coated endosomes containing NK₁ receptors, in their perinuclear translocation, in the resensitization of SP response, but not in MAPK activation. Neither dynamin, nor Rab5a are involved in NK₁ receptor desensitization (decrease SP-induced calcium mobilization upon repeated stimulation). Following agonist activation, NK₁r desensitizes, however, this process is not just the direct consequence of receptor stimulation, since desensitization is regulated by the N-terminal part of NK₁r agonists, as determined by structure–activity relationships [3]. Desensitization is mediated by several processes such as receptor phosphorylation, G-protein uncoupling, and receptor endocytosis. Different families of protein kinases are responsible for receptor phosphorylation. G-protein receptor kinases (GRKs) phosphorylate C-terminal threonine or serine residues of the receptor and favor its binding to beta-arrestins, thus promoting receptor uncoupling to G-proteins; since this phosphorylation only occurs when the agonist is associated to the receptor, this process is responsible

for the homologous desensitization. The other families of protein kinases involved in receptor desensitization are those activated by transduction mechanisms such as protein kinase A (PKA, cAMP dependent) and PKC (modulated by calcium and/or diacylglycerol): since Galpha-s and Galpha-q-mediated transduction mechanisms are shared by other kinds of G-protein coupled receptors, a cross-desensitization (heterologous desensitization) can occur through these kinases [2].

Receptor endocytosis is regulated by several mechanisms, one of which is the degree of glycosylation of the receptor itself. A double mutant of N-glycosylation sites in the extracellular tail of NK₁r (N14Q and N18Q) transfected in NCM 460 cells had a more rapid internalization kinetics upon SP stimulation as compared to wild type NK₁r. The binding affinity of SP and NKA as well as the ability of SP to promote phosphorylation of mitogen-activated protein kinases (MAPK) for the double mutant were not changed but modifications in receptor expression (decreased Bmax) and functional response (interleukin-8 secretion) were observed.

The binding of beta-arrestins to the receptors also induces receptor internalization in clathrin-coated vesicles, however, recent evidence also indicate that beta-arrestins are also involved in G-protein-independent receptor transduction mechanisms. Indeed, the activity of receptor-activated extracellular signal-regulated kinases 1 and 2 (ERK1/2) and MAPK can be increased both by conventional G-protein-mediated transduction mechanisms and beta-arrestin–receptor complexes independently from G-protein-mediated signaling. However, these two modalities of ERK1/2 activation differ in their time course since beta-arrestin-mediated activation is slower in the onset but longer lasting than that mediated by G-protein-mediated transduction mechanisms. Interestingly in a native system (human CaCo-2 cell line), SP increased the phosphorylation of MAPK without increasing intracellular calcium and the effect on MAPK was antagonized by the selective NK₁r antagonist CP-96345. In this system, the prototypical antagonist [D-Pro², D-Trp^{7,9}]-SP behaves as biased (or collateral) agonist (**biased agonism**), since it increased MAPK phosphorylation through the stimulation of NK₁r but it blocks SP-induced inositol phosphate accumulation in other systems [3].

TK NK₁r displays a broad distribution in both peripheral tissues and in the central nervous system (CNS). In both CNS and enteric neurons, NK₁r stimulation increase their excitability, whereas in trigeminal ganglion neurons SP has no intrinsic electrophysiological effects but is capable to enhance the amplitude of the inward current induced by the stimulation of serotonin 5-HT₃ receptors. This enhancement depends on the activation of PKC via the stimulation of NK₁ receptors. This is an interesting case of **receptor cross talk**. Other functions of NK₁r have been also highlighted.

SP, by acting through NK₁r located in inflammatory and immune cells, has proinflammatory effects and activate innate immunity. Furthermore, the peripheral release of TKs from ►capsaicin-sensitive primary afferent neurons, at the site of stimulation or at distal sites via an ►axon reflex, also plays a prominent role in initiating ►neurogenic inflammation, where the stimulation of NK₁r produces edema and in some districts participates in vasodilatation [3]. NK₁r located on glandular elements stimulate secretions (salivary and water intestinal secretion). SP acting at NK₁r can promote proliferation on both epithelial cells and fibroblasts, but in specific types of neurons can induce nonapoptotic, programmed cell death.

Tachykinin NK₂ Receptor

Two NK₂r splice variants have been identified (Table 3). Beyond the classic NK₂r, a splice variant (beta-NK₂r) lacking of several domains of the classic NK₂r (alpha-NK₂r) has been also described: however, no TK was capable to activate this splice variant, when transfected in CHO cells, since the receptor architecture was disrupted and the variant lacked domains containing critical amino acid residues for the binding of TKs [1]. NKA binding at NK₂r occurs through an interaction with residues of the N-terminal extracellular tail, the EC1 loop, the outer surface of TM3, TM4, and TM5 as well as aromatic residues of TM6 (see Fig. 1 for critical point mutations). Molecular dynamics of agonist-induced transduction mechanisms mediated by Galpha-q and Galpha-s have been clarified in a recombinant system by using fluorescent NKA and NK₂r labeled with fluorescent protein, thus, allowing simultaneous real time recording of ligand binding and calcium responses in living cells. Two phases of NKA binding to NK₂r were identified: a fast component showed a temporal and spatial correlation with intracellular calcium increase, whereas a slower component was associated with cAMP production and PKC-induced desensitization of the calcium response. In contrast, NKA(4–10) only displayed the fast component of the binding and selectively increased intracellular calcium [3]. An antagonist discriminating these two conformers of NK₂rs has been described: LPI805 behaved as a partial and noncompetitive inhibitor of the binding of NKA, it inhibited NKA-induced cAMP production but slightly enhanced NKA-induced calcium response. Indeed, a cross talk between these transduction pathways was outlined since PKC activation facilitated NKA-induced cAMP increase, whereas activation of PKA provided a feedback inhibition on NKA-induced cAMP increase. As for NK₁r, there is evidence of NK₂r-mediated activation of alternative transduction pathways. NK₂r-mediated synthesis of prostanoids was observed in both recombinant and native systems, and this effect was calcium dependent and SKF96365 sensitive but it was not affected by the phospholipase C

inhibitor U-73122, indicating that neither Galpha-q nor Galpha-s were involved. Interestingly, in a recombinant system expressing both NK₂r and transient receptor potential 3 (TRP3) channels, NK₂r agonists activated an inward cation current that was reduced by SKF96365 suggesting that NK₂r-triggered calcium entry responsible for prostanoid production is mediated by the activation of TRP3 channels. NK₂r stimulation also activated MAPK: this activation was transient and blocked by a cAMP analog, indicating the dependency from Galpha-s-mediated transduction. However, in a truncated form of the receptor lacking the intracellular C-terminal domain, NK₂r-induced MAPK activation was associated with increased DNA synthesis, and morphological changes such as cytoskeleton remodeling, cell growth, and transformation, effects which were not blocked by the cAMP analog. NKA-induced DNA synthesis, but not morphological changes, was reduced by ADP-ribosylation of Rho G-proteins, thus envisaging alternative signaling pathways in this system. It is worth noting that NK₂r-mediated cell proliferation is not restricted to this recombinant system but also occurs through the stimulation of native receptors expressed in breast cancer cell lines. If the stimulation of C-terminal truncated NK₂r induces cell proliferation, the activation of native NK₂r in hematopoietic stem cells inhibits cell proliferation through the activation of p53 protein. Although it is unclear which transduction pathway is responsible for p53 activation, these results highlight how the cellular environment is crucial for the final effect observed.

TK NK₂rs are mainly expressed in peripheral tissues, although a low level of expression has been detected in several CNS areas. The stimulation of these receptors induces contraction of airway, gastrointestinal, and genitourinary smooth muscle [3, 5]. In both airways and urogenital organs, NK₂r mediate muscle contraction that follows the activation of the ►efferent function of sensory nerves. In these systems, functional expression has been detected also in epithelial cells, where NK₂r activation increases the luminal permeability of hollow organs, effect that is probably mediated by nitric oxide. There is also evidence that visceral sensitivity is increased through the peripheral stimulation of NK₂r; since TKs are released from sensory nerves at the peripheral level (see above), this mechanism represents a positive feedback enhancing the afferent function of sensory nerves, a mechanism which is believed to participate in the pathogenesis of visceral pain.

Tachykinin NK₃ Receptors

Beyond the classic NK₃r, an mRNA splice variant has been also recently identified but no data on its expression and function are available yet, although the extensive deletion of critical regions renders unlikely its functionality (Table 3) [5]. NK₃r has been much less

characterized in term of ligand interactions, molecular dynamics, and transduction mechanisms than other TK receptors. Agonist binding at TK NK₃r occurs through residues located in EC2 and EC3 loops, TM2, TM4, and TM7 [1]. NK₃r stimulation by NKB activates Galph α -s and Galph α -q mediated transduction mechanisms, thus increasing cAMP and inositol triphosphate levels [1, 3, 5]. Like other TK receptors, an increase in intracellular calcium has been described, but it is unclear whether this is only due to Galph α -q coupling or other transduction mechanisms are also involved. In a recombinant system, NK₃r stimulation by [MePhe⁷]-NKB induces a rise of intracellular calcium and nitric oxide production via Galph α -q coupling since both effects were inhibited by thapsigargin (that means they are mediated by inositol triphosphate). NK₃r-induced phosphorylation of MAP kinases ERK1/2 is mediated by receptor coupling with beta-arrestins. In fact, upon stimulation, NK₃r-mediated responses (increase of intracellular calcium) rapidly desensitize and internalize through a beta-arrestins mediated process. However, in recombinant or native systems coexpressing both NK₁r and NK₃r, the stimulation of NK₁r prevents the homologous desensitization and the internalization of NK₃r by competing on a common pool of beta-arrestins at which NK₁r have a higher affinity than NK₃r [2]. This is an interesting example of TK $\blacktriangleright$ receptor cross talk. Molecular basis for cases of tachykinin cotransmission can be identified in tachykinin receptor cross talk.

NK₃rs are preferentially expressed in the CNS. At the peripheral level, NK₃rs have been localized in the enteric nervous system, nerve terminals, and autonomic ganglia, but also on other tissues. At the peripheral level, it remains to be explained the mismatch between NK₃r expression and the apparent lack of expression of NKB: there are, however, physiological conditions

where NKB is expressed and secreted by peripheral organs, such as placenta during pregnancy [1].

Drugs

The first generation of TK receptor antagonists were peptides designed on the backbone of natural TKs [1, 3]. These antagonists had several drawbacks such as low affinity, limited selectivity, metabolic instability, negligible bioavailability, and several of them also displayed nonspecific effects. In the 1990s, a number of selective peptide and nonpeptide antagonists with an improved profile were designed and developed. More recently, because of the frequent coexpression of different TK receptors on the same cell type and the functional interactions between TK receptors, that is, TK $\blacktriangleright$ cotransmission, nonselective antagonists have been also developed (Table 6).

Tachykinin NK₁ Receptor Antagonists

The discovery of nonpeptide NK₁r antagonists paralleled that on species-related differences of the affinity of these antagonists which never clearly emerged with peptide NK₁r antagonists. In fact, CP-96345, as well as several antagonists listed in Table 5, possesses high affinity at human and guinea pig NK₁r, whereas the affinity at rat or mouse NK₁r was markedly lower. The opposite pattern was observed with RP-67580 which displays a high affinity at rat or mouse but quite low at both human and guinea pig NK₁r [1, 3]. Species-related differences could be attributed to divergent residues in NK₁r sequences of these species. In fact, the replacement of rat residues (V116L and I290S) into the human sequence decreased the affinity of CP-96345 and increased that of RP-67580. Critical point mutations for the binding of antagonists to the human NK₁r are shown in Table 7 [1].

Tachykinins and their Receptors. Table 6 Examples of selective and non-selective tachykinin receptor antagonists

Receptor selectivity	Antagonist
NK ₁	CP-96345, CJ-11974 (ezlopitant), CP-99994, CP-122721, RP-100893 (dapitant), RP-67580, GR-82334, GR-203040, GR-205171 (vofopitant), L-732138, L-703606, L-733060, L-161664, L-754030 (MK-869, aprepitant), SR-140333 (nolpitantium), SSR-240600, LY-303870 (lanepitant), TAK-637, SDZNKT-343, CGP-49823, MEN-11467, WIN-51708, FK-888
NK ₂	MEN-10376, MEN-10627, MEN-11420 (nepadutant), MEN13918, MEN-15596, L-659877, SR-48968 (saredutant), SR-144190, GR-94800, GR-159897, UK-224671, NK-5807, M-274773, LPI-805
NK ₃	SR142801 (osanetant), SSR-146977, SB-223412 (talnetant), SB-235375, SB-222200, SB-218795, PD-154740, PD-157672
NK ₁ -NK ₂	MDL-105212A, MDL-105172A, DNK-333A, YM-44778, ZD-6021, SCH-205528, AVE-5883
NK ₁ -NK ₃	GR-138676
NK ₂ -NK ₃	SB-400238, SSR-241586
NK ₁ -NK ₂ -NK ₃	CS-003, SCH-206272

Tachykinins and their Receptors. Table 7 Critical receptor amino acid residues for the binding of selective NK₁, NK₂, or NK₃ receptor antagonists at the respective human TK receptors

Receptor	Antagonist	Critical mutations (>10 times lower affinity)
NK ₁	CP-96345	Q165A, S, N; H197A, Y, S, K; Y272A
	RP-67580	H197K; H265A; Y287A; Y287Q, W, S
	L-161664	Q165A; H265A; Y287S
NK ₂	SR-48968 (saredutant)	H198A; Y266A, S; Y289A, F
	MEN-11420 (nepadutant)	C167G; T171A; Y206A; W263A; Y266A, F; F270A; Y289A, T
	MEN-13918	I202F; W263A; Y266F; F270A; Y289F; Y289T
NK ₃	SR-142801 (osanetant)	M134L; A146R

Critical residues were identified through heterologous competition, or affinity of the radiolabeled antagonist to the point-mutated receptor.

TK NK₁r antagonists have displayed potential therapeutic effects in preclinical models of pain (including migraine), depression, anxiety, drug abuse, experimental autoimmune encephalopathy, asthma, pulmonary hypertension, emesis, urinary incontinence, inflammation, cystitis, pancreatitis, and nephritis [3]. The clinical efficacy of NK₁r antagonists has been tested in some of the abovementioned diseases. To date, a clinical benefit has been clearly demonstrated in the treatment of emesis which has led to registration of the centrally acting NK₁r antagonist aprepitant [3]. This antagonist also showed promising clinical results in bladder overactivity, whereas the same antagonist or other ones displayed inconsistent results in trials for depression and asthma, or they were clearly not effective in trials for pain or migraine.

Tachykinin NK₂ Receptor Antagonists

Although initially interpreted as evidence of expression of NK₂r subtypes, the existence of species-related difference in NK₂r pharmacology was already emerged during the characterization of peptide antagonists. In fact, it was evident that the affinity of MEN-10207 and MEN-10376 for the rat receptor was lower than that for guinea pig and human receptors, whereas the opposite pattern of affinity was observed for L-659877 [1]. A similar, or even a deeper gap in receptor affinity has been reported for a new series of nonpeptide antagonists such as MEN-13918 and MEN-15596 which possess very high affinity at human and guinea pig NK₂r and much lower affinity for rodent receptors, where one important residue for the high-affinity binding at human receptors is F202. Critical point mutations at the receptor protein for the affinity of other NK₂r antagonists are shown in Table 7.

TK NK₂r antagonists showed interesting activities in preclinical models of depression, anxiety, asthma, gastrointestinal dysmotility and hypersensitivity, and urinary incontinence [1, 3]. In clinical, NK₂r antagonists antagonize NKA-induced bronchoconstriction and NKA-induced intestinal dysmotility. However, a pilot trial in asthma failed to show any clinical benefit by

NK₂r antagonists. At the CNS level, the efficacy of NK₂r antagonists in depression and anxiety is currently being tested.

Tachykinin NK₃ Antagonists

The interest in NK₃r as therapeutic target rose later than that for the two other TK receptors. Indeed, a few peptide selective antagonists have been available (R-486, R-487) up to the discovery of nonpeptide antagonists [1, 3]. SR-142801 also displayed species-related affinity, being more active at human and guinea pig NK₃r than the rodent counterparts and critical residues for this discrimination were identified in M134, A146 [1]. Preclinical research has not clarified the therapeutic potentials of these antagonists which displayed activity in models of drug abuse, asthma and intestinal dysmotility. SR-142801 and SB-223412 have been subjected to clinical development: no information is available on the latter drug, whereas the former was unsuccessful in a pilot trial on levodopa-induced dyskinesia but showed promising results in schizophrenia.

Nonselective Tachykinin Antagonists

This area represents a new challenge because, if it is true that nonselective antagonists display a broader therapeutic potential than selective drugs, the same concept applies for potential to precipitate adverse events. The dual NK₁r and NK₂r antagonists DNK-333 or AVE5883 or the pan-antagonist CS-003 have been shown to antagonize NKA-induced bronchoconstriction in asthmatics in a more efficient manner than selective NK₂r antagonists. AVE5883 did not reduce antigen-induced bronchoconstriction and methacholine hyper-reactivity in asthmatics, but no other clinical result is known for this kind of antagonists.

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Tachyphylaxis

Tachyphylaxis is a loss of drug efficiency which develops in minutes or hours. Transmitter depletion and receptor desensitization are the basic mechanisms of this phenomenon.

► Tolerance and Desensitization

Tacrolimus

► FK506

► Immunosuppressive Agents

Tandem Mass Spectrometry

Tandem mass spectrometry (MS/MS) is a method for obtaining sequence and structural information by measurement of the mass-to-charge ratios of ionized molecules before and after dissociation reactions within a mass spectrometer which consists essentially of two mass spectrometers in tandem. In the first step, precursor ions are selected for further fragmentation by energy impact and interaction with a collision gas. The generated product ions can be analyzed by a second scan step. MS/MS measurements of peptides can be performed using electrospray or matrix-assisted laser desorption/ionization in combination with triple quadrupole, ion trap, quadrupole-TOF (time-of-flight), TOF–TOF or ion cyclotron resonance MS. Tandem

mass spectrometry of peptides provides amino acid sequence-specific ions and can be used to identify and characterize proteins.

► Proteomics

Tardive Dyskinesia

Tardive dyskinesia (TD) refers to a neurological syndrome caused by the long-term use of antipsychotic medications. It is characterized by repetitive involuntary movements of the face, limbs, or trunk. The incidence of tardive dyskinesia is greater with conventional antipsychotic drugs than atypical antipsychotic drugs, and clozapine may even have an ameliorative effect on established TD. There is no known treatment for TD, though dose reduction, discontinuation of the offending drug, or substituting with an atypical antipsychotic drug may be beneficial.

► Antipsychotic Drugs

Target of Rapamycin Encoded by: TOR1 and TOR2 in Yeast

► TOR Signalling

Targeted Cancer Therapy

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Synonyms

Molecular cancer therapeutics

Definition

Targeted cancer therapy refers to anticancer treatments that selectively interfere with molecules (► **oncogenes** and ► **antioncogenes**) considered to be important in neoplastic transformation, cell proliferation, invasion

and metastasis, and/or tumor-related angiogenesis [1]. Targeted cancer therapy is intended to spare normal cells, by contrast with “classical” cytotoxic agents that are relatively nonspecific. Targeted cancer therapy is rationally designed, while “classical” cytotoxic agents are usually discovered through the systematic screening of number of substances primarily issued from nature.

Basic Mechanisms

First targets that have been triggered for cancer treatment are estrogens, progesterone, and their receptors, leading to the concept of hormone therapy. Although hormone function was initially unknown, first hormone therapy was used by Sir Georges Beatson in 1896 when he performed a bilateral ovariectomy in five patients with advanced breast cancer and had three tumor shrinkages. Ovariectomy leads to the blockade of estrogen and progesterone synthesis, and thus to the inhibition of tumor progression in hormone receptor-overexpressing breast cancers. Interestingly, 10 years later, targets were identified by Marshall and Jolly who described the hormonal function of ovaries. In 1930, Decourmelle was the first to perform an ovarian irradiation leading also to tumor response. “Medicinal” hormone therapy arised only in the 1960s. Hormone therapy (or antihormone therapy should we say) is the most older targeted cancer therapy used in oncology. A Number of hormone therapies have been developed for hormone receptor-overexpressing breast cancer therapy:

- Chemical ovarian suppression with the use of ►LH-RH agonists.
- Competitive inhibitors of estrogen receptors, including ►selective estrogen receptor modulators, such as tamoxifene, and ►selective estrogen receptor down-regulators, such as fulvestran. The first ones competitively bind to estrogen receptors, whereas the later ones induce estrogen receptor degradation.
- ►Aromatase inhibitors that inhibit the androgen aromatization in estrogens and thus block estrogen synthesis.

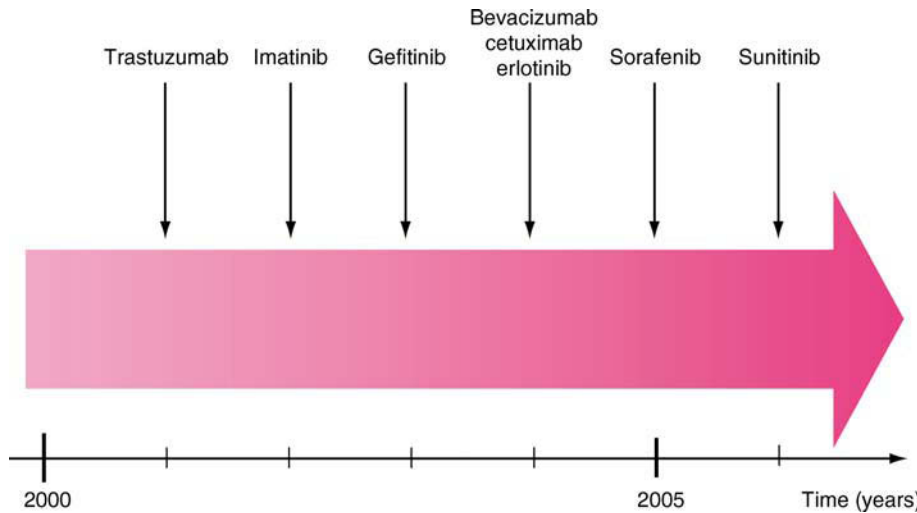
Besides the hormone therapy, the notion of targeted cancer therapy is not so new and can be traced back to the 1940's [2]. Early approaches included the largely unsuccessful use of antibodies, often conjugated with radioisotopes or toxins, directed against tumor-associated antigens.

More recently, novel targets to targeted cancer therapy have emerged and successfully been introduced in clinical practice. These therapies have been designed to target key components of signaling pathways important in malignant cell transformation and proliferation. Number of growth factor receptors, principally receptor tyrosine kinases, located on tumor cell membrane,

such as epidermal growth factor receptor (EGFR) (►epidermal growth factor receptor family), ►epidermal growth factor receptor 2 (HER-2), and KIT; on endothelial cells, such as ►vascular endothelial growth factor receptor (VEGFR); and on pericytes, such as ►platelet-derived growth factor receptor (PDGFR), have been identified. In each case, activation of the receptor tyrosine kinase causes autophosphorylation of the tyrosine kinase within its intracellular domain, which triggers a cascade of intracellular signals that ultimately promote malignant transformation and tumor progression. In addition, intracellular serine/threonine kinases have also been identified as key kinases in signaling pathways leading from receptor tyrosine kinase activation to cell proliferation and other processes important for cancer development and progression. Three major signaling pathways have been identified as playing important roles in cancer, including mitogen-activated protein kinase (MAPK)/Ras, the phosphatidylinositol-3-kinase (PI3K)/AKT, and the protein kinase C (PKC) signaling cascades. A number of drugs in development inhibit multiple receptor tyrosine kinases and/or intracellular serine/threonine kinases at the convergence of multiple signaling pathways. The critical idea underlying targeted cancer therapy is that excessive activity of the receptor tyrosine kinase promotes cancer development and progression, and thereby inhibition of receptor tyrosine kinases may break this pathogenic chain. The beginning of the twenty-first century has been a turning point in oncology with approvals of several targeted cancer therapies for the treatment of patients with solid tumor treatment (Fig. 1).

Targeted cancer therapy that first entered clinical practice were tyrosine kinase inhibitors, including monoclonal antibodies and small-molecule tyrosine kinase inhibitors. These drugs were specifically designed to inhibit one key cellular protein associated with tumor development and progression. Monoclonal antibodies bind either to the extracellular domain of transmembrane tyrosine kinase receptors or directly to their ligands, thereby preventing the endogenous ligand from binding and activating the receptor. Monoclonal antibodies have a single target, and required to be administered intravenously. Three monoclonal antibodies have been successfully introduced in clinical practice for the treatment of solid tumors, including trastuzumab, bevacizumab, and cetuximab (Table 1).

- Trastuzumab specifically binds the extracellular domain of HER-2 that is found overexpressed on the membrane of cancer cells in 15–20% of patients with breast cancer. It has been approved in HER-2 overexpressing (HER-2 +++ or FISH +) breast cancers.



Targeted Cancer Therapy. Figure 1 History of approval of targeted cancer therapy in clinical practice.

Targeted Cancer Therapy. Table 1 Approved targeted cancer therapies in clinical practice

Molecule	Company	Type	Target(s)	Approved indication(s)
<i>Trastuzumab</i> (Herceptin [®])	Roche	mAb	HER-2	HER-2 overexpressing breast cancer in the adjuvant and metastatic settings
<i>Imatinib</i> (Gleevec [®])	Novartis	TKI	KIT, PDGFR, BCR-ABL	KIT overexpressing unresectable or metastatic gastrointestinal stromal tumor
<i>Gefitinib</i> (Iressa [®])	Astra-Zeneca	TKI	EGFR	Metastatic non-small cell lung carcinoma
<i>Bevacizumab</i> (Avastin [®])	Roche	mAb	VEGFR	Metastatic colorectal cancer Metastatic non-small cell lung carcinoma
<i>Cetuximab</i> (Erbix [®])	Merck	mAb	EGFR	Metastatic colorectal cancer Advanced head and neck cancer
<i>Erlotinib</i> (Tarceva [®])	Roche	TKI	EGFR	Metastatic non-small cell lung carcinoma Metastatic pancreatic cancer
<i>Sorafenib</i> (Nexavar [®])	Bayer	TKI	VEGFR, PDGFR, KIT, FTL-3, RAF	Unresectable or metastatic renal cell carcinoma
<i>Sunitinib</i> (Sutent [®])	Pfizer	TKI	VEGFR, PDGFR, KIT, FLT-3, RET	Imatinib-resistant KIT overexpressing unresectable or metastatic gastro-intestinal stromal tumor Unresectable or metastatic renal cell carcinoma

mAb: monoclonal antibody; TKI: tyrosine kinase inhibitor.

- Bevacizumab specifically binds VEGF, present in tumor vasculature, and has been designed to disrupt tumor angiogenesis. Angiogenesis is an ideal anti-cancer target because in healthy adults it is restricted to a few short episodes, so that antiangiogenic therapy tends to spare normal tissues. Moreover, vascular endothelial cells are genetically more stable and less likely to develop rapid resistance to therapy than neoplastic cells. They are also a relatively uniform cell type, so that antiangiogenic therapy is potentially effective across a broad spectrum of tumors. Bevacizumab has been approved in colorectal and non-small cell lung cancers.

- Cetuximab specifically binds the extracellular domain of EGFR. It has been approved in colorectal and head and neck cancer. However, unlike for trastuzumab, no clear correlation has been demonstrated between EGFR expression in cancer cell and efficacy of cetuximab.

Interestingly, all these monoclonal antibodies have a poor antitumor activity when used as single agents, with overall response rates of about 10%. Thereby, these monoclonal antibodies have all been approved in combination with “classical” cytotoxic agents or radiation therapy.

Small-molecule tyrosine kinase inhibitors that bind to the intracellular domain of transmembrane receptor tyrosine kinases may have one or several targets and, due to the lack of solubility, are orally administered. Small-molecule tyrosine kinase inhibitors that have been approved in clinical practice include gefitinib, erlotinib, imatinib, sorafenib, and sunitinib, the first two being the only ones that have a single target, and the later being considered as multitargeted agent (Table 1).

- Erlotinib and gefitinib selectively bind EGFR. Gefitinib has been approved in non-small cell lung cancer, while erlotinib has been approved in non-small cell lung and pancreatic cancers. Imatinib that selectively binds ►BCR-ABL fusion protein, KIT, and PDGFR, is the first approved targeted cancer therapy that may be considered to be a multitargeted tyrosine kinase inhibitor. BCR-ABL has been linked with chronic myeloid leukemia, while KIT and PDGFR have been associated with gastrointestinal stromal tumors (GIST). Thus, imatinib has been approved in chronic myeloid leukemia and GIST.
- Sorafenib is a multitargeted cancer therapy that inhibits VEGFR, PDGFR, KIT, fetal liver tyrosine kinase 3 (FLT-3), and the serine/threonine kinase RAF. RAF kinase is a key downstream effector of Ras in the MAPK/Ras signal-transduction pathway that has been linked to various cancers. Sorafenib is both a tyrosine kinase inhibitor and serine/threonine signal-transduction inhibitor. Sorafenib has been approved in renal cancer.
- Sunitinib is also a multitargeted cancer therapy targeting VEGFR, PDGFR, KIT, FLT-3, and RET. Sunitinib has been approved in GIST refractory to imatinib and renal cancer.

Main differences between monoclonal antibodies and small-molecule receptor tyrosine kinases are described in Table 2.

In addition to the targeted cancer therapies described above, other agents in development have been designed to target downstream signaling effectors, i.e., molecules downstream from the receptor tyrosine kinase that are part of the signaling cascades leading to cancer development and progression. These latter agents are sometimes referred to as signal-transduction inhibitors. Some of these agents, like the mammalian target of rapamycin (mTOR) inhibitors, have been designed to affect a key molecule at the convergence of signaling cascades generated by various receptor tyrosine kinases. PKC is another important molecule in signal-transduction pathways, and PKC inhibitors are in development as potential new targeted cancer therapy. Both mTOR and PKC inhibitors target serine/threonine protein kinases.

Targeted Cancer Therapy. Table 2 Main differences between monoclonal antibodies and small-molecule tyrosine kinase inhibitors

Monoclonal antibodies	Small-molecule tyrosine kinase inhibitors
Intravenous administration no absorption problem	Oral administration submitted to absorption variations
Few submitted to metabolism variations, in particular to hepatic metabolism	Submitted to metabolism variations, in particular via cytochromes
Long half-life (>1–2 weeks)	Short half-life (<48 h)
Few pharmacokinetic variations	Possible pharmacokinetic variations
Limited tissular penetration	Satisfactory tissular penetration

Pharmacological Relevance

Many of the initial targeted cancer therapies were focused on a single oncogene product that was found important for cancer development and progression, typically focusing on either molecules associated with direct cancer cell proliferation or those associated with tumor angiogenesis. Increasingly, there is an understanding that monotherapy with agents focused on a single target may produce less than optimal results. In 2000, Hanahan and Weinberg published their theory on tumor growth, suggesting that there are six essential processes that play key roles in tumor growth: self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasive and metastasis [3]. Moreover, they noted that malignant tumors are complex tissues that involve interactions between cancer and supporting cells, including vascular endothelial cells, fibroblasts, and immune cells. Pericytes (which express PDGFR) may also play important roles in tumor-related angiogenesis.

For maximal benefit, it seemed logical that more than one cancer-related target may need to be inhibited [4]. This may involve combining targeted therapies with “classical” cytotoxic agents, as it is the case for monoclonal antibodies, or using targeted agents with more than one target. These latter multitargeted cancer therapies have the benefit of minimizing the number of agents that patients would be required to take. Theoretically, multitargeted cancer therapies could also be combined with “classical” cytotoxic agents for maximal benefit. Imatinib, sorafenib, and sunitinib are currently the only members of this class on the market, but a number of investigational products are in development that may soon be available.

There are a number of reasons for expecting multitargeted cancer therapy to be superior to targeted cancer therapy focused on a single target or to “classical,” nonselective cytotoxic agents.

- For one, cancer development is characterized by multiple abnormalities rather than a single defect, and it is unlikely that a single-target agent will have dramatic effects on both cancer cells and cells supporting tumor development, such as endothelial cells and pericytes. Thus, maximally effective therapy may need to involve a multitargeted therapy or combination of drugs targeting signaling pathways in both tumor cells and endothelial cells or pericytes. Additionally, one may expect that targeting two components in a given cancer-related pathway will have greater effects than targeting a single component in the pathway. This is another potential argument for targeting of multiple molecules important for cancer development.
- Moreover, by the time a tumor has grown to any significant extent, it has become heterogeneous and is likely to contain resistant clones to single-target cancer therapy. Larger tumors contain cells that have undergone several cell divisions, which increase the odds of mutations, including those associated with drug resistance. This is one reason why more advanced solid tumors commonly become refractory to treatment with single-target agents or “classical” cytotoxic agents using a single mechanism of action. Additionally, new resistance-conferring mutations may arise over time. In either case, resistance is less likely to be an issue when therapeutic regimens target multiple molecules involved in cancer development or utilize multiple mechanisms of action. Thus, the other major argument for multitargeted or combination cancer therapy is increased likelihood of sustained effectiveness compared with treatment involving single-target drugs or agents using a single mechanism of action.
- Finally, the effectiveness of “classical” cytotoxic agents may increase when combined with targeted cancer therapies. In the case of agents targeting angiogenesis, the improvement may be attributed, at least in part, to an initial normalization of the tumor vasculature that results in improved delivery of “classical” cytotoxic agents to the tumor target. In some cases, resistance to cytotoxic therapies may be overcome or prevented in the first place when used in combination with targeted cancer therapies.

Many of these strategies, particularly the ones involving different drug combinations, are largely theoretical at this point and require validation of the concept and determination of optimal regimens. Increased understanding of the mechanisms underlying cancer

suggests that an integrated approach to cancer therapy involves inhibition of multiple signaling pathways.

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Taste Receptors

Sensory receptors expressed in particular in taste receptor cells of the taste buds that sense the five basic tastes: salt, sour, sweet, bitter and umami (glutamate taste). Sodium type ion channels sense salty taste whereas sour taste is transduced by potassium type ion channels. The underlying cause of sweet, bitter, and umami tastes is the selective activation of different groups of G protein coupled receptors that discriminate between sweet, bitter, and umami tasting molecules.

► Orphan Receptors

TATA Box

Consensus sequence in the promoter region of many eukaryotic genes that bind a general transcription factor and hence specifies the position where transcription is initiated by the RNA polymerase.

► Interferons

Taxanes

Anticancer drugs originally extracted from the cortex of *Taxus brevifolia* or *Taxus baccata*. They block cell division by inhibiting tubulin depolymerization. Two

taxanes, paclitaxel and docetaxel, have been approved for clinical use.

► **Antineoplastic Agents**

TCR

► **T Cell Receptors**

TDM

Therapeutic drug monitoring by measurement of concentrations at a defined time related to drug dosing.

► **Pharmacokinetics**

Temporal Lobe

The temporal lobe is the inferior middle portion of the cerebral cortex of both hemispheres. The temporal lobes are involved in the analysis of visual and acoustic information and in memory formation. The hippocampus is part of the inner, medial side of the temporal lobes.

► **Antiepileptic Drugs**

Tendinitis

Tendinitis is an inflammatory painful tendon disorder which can be caused by quinolones. Typical cases are characterized by acute onset, palpation and sharp pain mostly of one or both Achilles tendons, but other tendons may also be affected. Magnetic resonance imaging (MRI) is used to support the diagnosis. Estimates for the incidence of quinolone-induced tendinitis range from approximately 1:100 to 1:10,000. The etiology remains unknown, concomitant

use of corticosteroids, end stage renal disease and age older than 60 years are recognized as risk factors. The latency period ranges from 2 to 60 days after start of treatment, but tendon ruptures may occur as late as 6 months after cessation of therapy.

► **Quinolones**

Teratogen

A teratogen (the word is of Greek origin, meaning the induction of a monster) is a chemical, physical or viral factor causing birth defects. Exposure to teratogens can result in a wide range of structural abnormalities.

► **Antimetabolites**

Testosterone

► **Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor**

Tetanus

Tetanus is a disease caused by the release of neurotoxins from the anaerobic, spore-forming rod *Clostridium tetani*. The clostridial protein, tetanus toxin, possesses a protease activity which selectively degrades the pre-synaptic vesicle protein synaptobrevin, resulting in a block of glycine and γ -aminobutyric acid (GABA) release from presynaptic terminals. Consistent with the loss of neurogenic motor inhibition, symptoms of tetanus include muscular rigidity and hyperreflexia. The clinical course is characterized by increased muscle tone and spasms, which first affect the masseter muscle and the muscles of the throat, neck and shoulders. Death occurs by respiratory failure or heart failure.

- **Bacterial Toxins**
- **Exocytosis**
- **Glycine Receptors**

- ▶ Ryanodine Receptor
- ▶ Synaptic Transmission

Tethered Ligand

A tethered ligand is the new N-terminal formed following serine proteinase-mediated cleavage of the original N-terminal of a PAR family member, which is responsible for activation of the receptor.

- ▶ Proteinase-Activated Receptors

Tetracycline

Tetracycline is an antibiotic with a flat structure of four fused rings with hydrophilic groups on one side and hydrophobic groups on the other side. It exhibits a broad spectrum of activity against Gram-positive and Gram-negative bacteria. It has been used widely in human and veterinary medicine until the recent spread of antibiotic resistance. Resistance to tetracycline, unlike many other antibiotics, is not caused by mutation of ribosomal RNA (rRNA) or ribosomal proteins or modification of a rRNA target site. It is rather caused by enzymes that result in (i) efflux of the drug across the cell membrane, (ii) modification of the drug or (iii) mimicry of elongation factors and release of the bound drug from the ribosome.

- ▶ Ribosomal Protein Synthesis Inhibitors

Δ^9 -Tetrahydrocannabinol (THC)

THC is the most abundant and most active cannabinoid found in the hemp plant ▶ *Cannabis sativa*. It constitutes about 1–10 % of *Cannabis sativa* preparations like marijuana or hashish.

- ▶ Endocannabinoids

3',5',3,5-L-Tetraiodothyronine

- ▶ Thyroxine

Tetramer

In the present context, a tetramer is a symmetric arrangement of four subunits, which generates K channel. Nearly all known K channels are tetramers of identical or similar alpha subunits often associated with additional beta subunits arranged again in a tetrameric fashion. Some K channels are dimers, but each part of their alpha subunits represents a two-fold repeat of a monomer.

- ▶ Inwardly Rectifying K⁺ Channels

Tetrodotoxin

Tetrodotoxin (TTX) is a toxin derived from bacteria which is concentrated in the gonads and liver of certain pufferfishes (fugu). Similar to saxitoxin, tetrodotoxin is a very potent blocker of most voltage-sensitive Na⁺ channels.

- ▶ Voltage-dependent Na⁺ Channels

TF

- ▶ Tissue Factor

Tg

- ▶ Thyroglobulin

TGF- β Receptor Signaling Pathway

- Transforming Growth Factor-Beta

TH

- Tyrosine hydroxylase

Thalamocortical Synchronisation

The synchronised oscillatory activity between the intrinsically linked thalamus and cortex. Under normal circumstances there is a level of activity which changes during the sleep–wake cycle increasing during periods of slow wave sleep. Excess synchrony occurs in conditions such as epilepsy.

- Sleep

THC

- Δ^9 -Tetrahydrocannabinol (THC)

Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) is advised for the individualization of dosage of drugs with a narrow therapeutic index and broad inter-individual pharmacokinetic variability. This is routinely done by determination of blood levels.

- Pharmacogenetics
- Pharmacokinetics

Thiamin

- Vitamin B1

Thiazide Diuretics

Thiazide diuretics, a group of drugs with moderate diuretic activity, includes hydrochlorothiazide, chlorthalidone, and xipamide. They decrease active reabsorption of sodium and accompanying chloride by binding to the chloride site of the electroneutral Na^+/Cl^- cotransport system in the distal convoluted tubule and inhibiting its action.

- Diuretics
- Epithelial Na^+ Channel

Thiazolidinedione

A class of drug that were developed as insulin-sensitizing agents and are currently used for the treatment of type 2 diabetes. They have been shown to bind to and activate the γ isoform of the PPARs, which is particularly expressed in adipocytes, and appear to function in part by stimulating the release of adiponectin from those cells.

- Antidiabetic Drugs
- AMP-activated Protein Kinase
- Adipokines
- PPARs

Thienopyridines

Thienopyridines are a Group of antiplatelet agents including ticlopidine and clopidogrel, which after oral administration are converted to active metabolites. The active metabolites in turn are able to block the P_2Y_{12} -receptors, which physiologically mediate part of the action of ADP on platelets.

- Antiplatelet Drugs

Thionamides

► Antithyroid Drugs

Thiopurine S-methyltransferase

Thiopurine S-methyltransferase is an enzyme which inactivates the anticancer drug 6-mercaptopurine by S-methylation.

► Pharmacogenetics

Thioxanthene

Thioxanthenes are a group of antipsychotic drugs (e.g. chlorprothixene).

► Antipsychotic Drugs

Threading

Threading techniques try to match a target sequence on a library of known 3D structures by “threading” the target sequence over the known coordinates. In this manner, threading tries to predict the 3D structure starting from a given protein sequence. It is sometimes successful when comparisons based on sequences or sequence profiles alone fail due to a too low sequence similarity.

► Bioinformatics

Thrifty Gene Hypothesis

The thrifty gene hypothesis postulates that specific sets of genes, which optimized energy utilization and storage, prepared our ancestors for “feast and famine” by efficiently protecting energy reserves when supplies

were low and by rapidly replenishing them when supplies increased again. With unlimited food resources, the thrifty gene haplotype is responsible for a metabolic syndrome of obesity, insulin resistance, hypertension, and dyslipidemia. This dependence of the phenotype on “lifestyle” was first observed in Polynesian and American Indian populations.

► Antiobesity Drugs

Thrombin

Thrombin (factor IIa) is an enzyme of 295 amino acids derived from prothrombin (vitamin K-dependent zymogen) that converts soluble fibrinogen into insoluble fibrin; other procoagulant activities of thrombin include activation of factor XIII to factor XIIIa (which irreversibly crosslinks fibrin polymers) and activation of the nonenzymatic coagulation factors V and VIII to Va and VIIIa, respectively (greatly amplifying thrombin generation). Thrombin also activates platelets. In addition, thrombin binds to thrombomodulin on endothelium, which causes thrombin to lose its procoagulant activities, and instead to convert protein C to activated protein C (APC), which downregulates thrombin generation by proteolyzing factors Va and VIIIa.

- Anticoagulants
- Coagulation/Thrombosis
- Proteinase-activated Receptors
- Vitamin K

Thrombin Receptors

► Proteinase-activated Receptors

Thrombocytopenia

Thrombocytopenia is a decrease in the number of circulating blood platelets (below $150 \times 10^9/L$). Although severe thrombocytopenia can lead to

spontaneous bleeding, a few disorders (e.g., heparin-induced thrombocytopenia) are paradoxically associated with an increased risk of thrombosis.

- ▶ Anticoagulants
- ▶ Hematopoietic Growth Factors
- ▶ Antiplatelet Drugs

Thrombolysis

Thrombolysis is fibrinolysis.

- ▶ Fibrinolytics

Thrombopoietin

- ▶ Hematopoietic Growth Factors

Thrombosis

Thrombosis is the development of a “thrombus”, consisting of platelets, fibrin, red and white blood cells in the arterial or venous circulation. Platelet-rich “white thrombi” are found in the arterial system and can be prevented by antiplatelet drugs.

- ▶ Anticoagulants
- ▶ Antiplatelet Drugs
- ▶ Coagulation/Thrombosis
- ▶ Fibrinolytics
- ▶ Purinergic System

Thromboxane

Prostanoids.

- ▶ Cyclooxygenase

Thrombus

A thrombus is a mass of cells and protein composed principally of platelets and fibrin, but also containing red and white blood cells. A thrombus which forms in the circulatory system can become occlusive in that the thrombus can physically block flowing blood.

- ▶ Thrombosis

Thymoleptics

- ▶ Antidepressant Drugs

Thymus

The thymus is a lympho-epithelial organ, located within the upper thorax. One of its functions is the conversion of certain lymphoid hematopoietic precursor cells originating from the bone marrow into thymus-derived lymphocytes (T-cells).

- ▶ T Cell Receptors

Thyroglobuline

Thyroglobulin (Tg) provides the matrix for thyroid hormone biosynthesis. It is a dimeric glycoprotein with a molecular weight of 660,000. Most of the thyroglobulin is present in the thyroid follicular lumen. Thyroglobulin contains 0.1–2.0% iodine and, as a glycoprotein, it contains 8–10% total carbohydrate, with galactose, mannose, fucose, *N*-acetyl glucosamine and sialic acid residues. The carbohydrate in the protein is distributed as three distinct units, A, B and C. Tg is synthesized on polysomes on the endoplasmic reticulum near the basal portion of the cell. After phosphorylation and glycosylation it is vectorially transported to

the apical membrane of the cell and deposited into the follicular lumen.

► Antithyroid Drugs

Thyroid Autonomy

Thyroid autonomy appears as a solitary toxic nodule or toxic multinodular goitre. In toxic thyroid, the nodule's synthesis and secretion of thyroid hormones is autonomous from the thyroid-stimulating hormone (TSH), which is produced in the pituitary gland. Accordingly TSH is suppressed and the extranodular thyroid tissue is functionally downregulated. Thyroid autonomy occurs frequently in iodine-deficient countries, whereas it is much less common in iodine-sufficient areas. Constitutively activating mutations in the TSH receptor and in the Gs α protein are the major molecular aetiology of toxic thyroid nodules.

► Antithyroid Drugs

Thyroid Peroxidase

Thyroid peroxidase (TPO) is a membrane-bound, glycosylated, hemoprotein enzyme. It catalyzes iodination of tyrosyl residues and the coupling of iodotyrosyl residues in thyroglobulin to form thyroxine and triiodothyronine. All mammalian peroxidases belong to the same gene family. The human TPO gene consists of 17 exons and 16 introns and covers at least 150 kb pairs. The heme iron in native peroxidases including TPO and LPO, is in the ferric form (Fe III). In model systems, TPO has no catalytic activity in the absence of H_2O_2 and there is little doubt that H_2O_2 production plays an essential role in thyroid hormone formation in vivo. The H_2O_2 generating system is Ca^{2+} dependent and involves a nicotinamide ADPH (NADPH) oxidase.

► Antithyroid Drugs

Thyrotoxicosis

► Hyperthyroidism

Thyroxine

Thyroxine (3',5',3,5-L-teraiodothyronine, T₄) is a thyroid hormone, which is transformed in peripheral tissues by the enzyme 5'-monodeiodinase to triiodothyronine. T₄ is 3–8 times less active than triiodothyronine. T₄ circulates in plasma bound to plasma proteins (T₄-binding globulin, T₄-binding prealbumin and albumin). It is effective in its free non-protein-bound form, which accounts for less than 1%. Its half-life is about 190 h.

► Antithyroid Drugs

TIMP

The matrix metalloproteinases are inhibited by specific endogenous tissue inhibitor of metalloproteinases (TIMPs), which comprise a family of four protease inhibitors: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. Overall, all MMPs are inhibited by TIMPs once they are activated but the gelatinases (MMP-2 and MMP-9) can form complexes with TIMPs when the enzymes are in the latent form.

► Matrix Metalloproteinases

TIR Domain

Toll/IL-1 receptor domain – A domain found on the internal C-terminus of Toll-like receptors involved in binding the adapter proteins to initiate signalling inside the cell.

► Toll-like Receptors

Tissue Factor

Tissue factor (TF) is an integral membrane glycoprotein, which is tightly associated with phospholipid (molecular weight 44 kDa). It is located on most

vascular cells and by functioning as a receptor for factor VII, initiates the extrinsic pathway of the blood coagulation cascade. The formation of a 1 to 1 complex with factor VII in the presence of Ca^{2+} -ions facilitates the conversion of factor VII to factor VIIa by minor proteolysis.

- ▶ Coagulation
- ▶ Anticoagulants

Tissue-specific Agonists/Antagonists

- ▶ Selective Sex Steroid Receptor Modulators

Tissue-type Plasminogen Activator

Tissue-type plasminogen activator (tPA) is a glycoprotein (68 kDa), synthesized by endothelial and tumor cells. As a serine protease, tPA hydrolyses $\text{Arg}_{561}\text{--Val}_{562}$ peptide bond in plasminogen, resulting in plasmin formation. It needs cofactors for efficient plasminogen activation.

- ▶ Fibrinolytics

T-kinin (Ile-Ser-Bradykinin)

- ▶ Kinins

TKIs

- ▶ Tyrosine Kinase Inhibitors

TLR

Toll-like receptor – Transmembrane proteins involved in the recognition of components of pathogens that

are not found in the host to initiate an innate immune response.

- ▶ Toll-like Receptors

T-lymphocytes

T-lymphocytes are hematopoietic cells belonging to the adaptive immune system. The prefix “T” indicates that immature precursors migrate to the thymus and are released as mature T-cells. During the maturation process in the thymus, a selection process eliminates both inactive and self-reactive cells. Each T-cell displays a specific receptor for antigen. From a functional point of view, T-cells are classified into cytokine-producing CD4^+ or helper T-cells that provide growth and activation factors to neighboring cells, and CD8^+ or cytotoxic T-cells, that specifically attack and kill target cells expressing the specific antigen. The composition of cytokines released by CD4^+ cells allows a classification into Th1 and Th2 populations. The term “Memory cells” describes a population of sensitized T- or B-cells that persist in the host and can rapidly be reactivated upon exposure with the specific antigen.

- ▶ Immune Defense
- ▶ Immunosuppressive Agents

TNF

Tumor Necrosis Factor.

- ▶ Tumor Necrosis Factor α
- ▶ TNF Receptor Superfamily
- ▶ Cytokines

TNF Receptor Associated Factors

Tumor necrosis factor receptor associated factors are a group of at least 6 proteins (TRAF1-6), which are major signal transducers for the TNF receptor superfamily and the interleukin-1 receptor/toll-like receptor (IL-1R/TLR) superfamily. TRAF proteins are characterized by the presence of the TRAF domain at the C-terminus,

which mediates self-association and upstream interaction with receptors and other signaling proteins. They function as adaptor proteins, which elaborate receptor signal transduction by serving as both a convergent and divergent platform.

- ▶ Tumor Necrosis Factor α
- ▶ TNF Receptor Superfamily
- ▶ Cytokines
- ▶ Inflammation
- ▶ Table appendix: Receptor Proteins

TNF Receptor Superfamily

The TNF receptor superfamily is a group of receptors, which share some structural similarities. Best-studied examples are the receptors for tumor necrosis factor α (TNF α) and the Fas receptor. Binding of the inherently trimeric ligands induces receptor trimerisation and results in the recruitment of several signaling proteins to the cytoplasmic domains of the receptors. TNF receptors recruit the “TNFR1-associated death domain protein” (TRADD), which serves as a platform to recruit several other mediators including “receptor-interacting protein 1” (RIP1), “Fas-associated death domain protein” (FADD) and “TNF-receptor associated factor 2” (TRAF2). TRAF2 plays a central role in the induction of downstream events like activation of I κ B-kinase (IKK) and MAP-kinases. Activation of Fas receptor does not result in the recruitment of TRAF2, but recruits FADD and subsequently leads to activation of caspase 8.

- ▶ Apoptosis
- ▶ Tumor Necrosis Factor α
- ▶ TNF Receptor Associated Factors
- ▶ Cytokines
- ▶ Inflammation
- ▶ Table appendix: Receptor Proteins

Tocolytics

Tocolytics are drugs such as the selective β_2 -adrenoceptor agonists, e.g. salbutamol or terbutaline, which inhibit both the spontaneous and oxytocin-induced contractions of the pregnant uterus by relaxing

the muscles of the uterus (myometrium). Tocolytics are used in selected patients to prevent premature labor.

- ▶ β -Adrenergic System
- ▶ Smooth Muscle Tone Regulation

Tolerance

The term tolerance is used to describe a gradual decrease in responsiveness to a drug, typically developing over days or weeks. A fast loss of responsiveness developing, e.g., over a few minutes is called desensitization or tachyphylaxis. It is important to note that tolerance does not only develop with drugs of abuse but also after repeated administration of a wide variety with drugs that are not self-administered by animals or used compulsively by man. Examples of the phenomenon of tolerance to opioids are described. Opioid tolerance is not associated with changes in affinity and/or density of opioid receptors. Opioid receptor desensitization is also not associated with receptor internalization but underlies uncoupling of the G protein.

- ▶ Drug Addiction/Dependence
- ▶ Tolerance and Desensitization

Tolerance and Desensitization

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Definition

▶ **Tolerance** is the reduction in response to a drug after repeated administration. Clinically, a higher dose is required to obtain the original response. The time course and extent of tolerance developments varies between different drugs. At least two types of tolerance, ▶ **pharmacokinetic** and ▶ **pharmacodynamic tolerance**, can be distinguished. Tolerance development is a reversible process. The phenomenon of ▶ **cross tolerance** may develop to the effect of pharmacologically-related drugs, particularly to those acting at the same receptor. Drug tolerance can involve not only physiological but also psychological factors.

► **Desensitization** describes the rapid signal attenuation in response to stimulation of cells by receptor agonists. Changes in the coupling efficiency of receptors to signal transduction pathways and receptor internalization can account for ► **desensitization** and the development of pharmacodynamic tolerance.

Basic Mechanisms Removal of the Agonist

The action of an agonist can be terminated by its removal from the extracellular fluid. This includes simple dissociation and diffusion as well as the reuptake and metabolic inactivation of the agonist. Latter mechanisms are fast and powerful and underlay a fine-tuned regulation that can adapt its capacity to maintain a physiological homeostasis. Many clinically used agonists are inactivated by similar mechanisms as the physiological ligands. Therefore, continued agonist treatment can induce an increased drug degradation. Such mechanisms may contribute to the development of a pharmacokinetic tolerance. For example, barbiturates induce their own metabolic inactivation in the liver, a process that leads to a reversible pharmacokinetic tolerance. Chemical modifications of an agonist that prevent reuptake and metabolic degradation can help to circumvent this form of tolerance.

Receptor Desensitization

Receptor desensitization summarizes pharmacodynamic processes that lead to an inactivation of receptor signaling within seconds to minutes. The rapid attenuation of extracellular signals mediated by G protein-coupled receptors (GPCRs) and their signaling pathways belong to the best-studied systems. The uncoupling of the receptor from its G protein involves receptor phosphorylation by different classes of protein kinases. Two types of receptor desensitization can be distinguished. ► **Homologous desensitization** is mediated by agonist-induced activation of the same receptor, whereas ► **heterologous desensitization** is caused by activation of a different receptor. The so-called ► **G protein-coupled receptors kinases** (► **GRKs**) are responsible for homologous agonist-induced receptor phosphorylation. So far, seven GRKs have been cloned. The expression of GRK1 and -7 is confined to retinal rods and cones, respectively. GRK2, GRK3, GRK5, and GRK6 are the most widely distributed GRKs, but expression and knockout studies also implicate more distinct roles of individual GRKs. Following receptor activation by an agonist, GRKs translocate from the cytosol to the plasma membrane. But there are also GRKs (GRK4, 5, 6) which constitutively associate with membranes. The signal which recruits GRKs to the activated receptor is not fully understood. Changes in the three-dimensional structure of the intracellular receptor surface, exposition of the phosphorylation consensus sites as well as a direct interaction of

GRKs and G proteins are discussed. The sites (mainly serine residues) phosphorylated by GRKs are located within the third intracellular loop and the intracellular C terminus of GPCRs. Once the receptor is phosphorylated, the functional cofactors of GRKs, the ► **arrestins** bind to the GPCR and thereby quench the signal transduction by disrupting the interaction of the receptor and the G protein. At least four arrestins – the visual arrestin 1 and arrestin 4 and the ubiquitously expressed arrestins 2 and 3 (also known as β -arrestin-1 and β -arrestin-2) – can be distinguished. β -Arrestins are essential for ► **internalization** of many GPCRs and some other membrane receptors (TGF- β type III receptor, IGF1 receptor). They act as adaptor proteins that link the receptors to the clathrin-coated pit endocytosis pathway (see Receptor Internalization).

Second messenger-dependent kinases such as protein kinase A (PKA), protein kinase C (PKC) and Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) are mainly involved in heterologous agonist-independent receptor desensitization. The heterologous desensitization includes receptors that use similar signal transduction pathways and simply depends on the overall kinase activity regulated by many different stimuli. Phosphorylation-induced conformational changes of the receptor reduce the affinity to the G protein, thereby leading to receptor/G protein uncoupling.

Many GPCRs contain one or more conserved cysteine residues within their C-terminal tails, which are modified by covalent attachment of palmitoyl or isoprenyl residues. The palmitoyl moiety is anchored in the lipid bilayer forming a fourth intracellular loop. There is evidence that palmitoylation of a GPCR is a dynamic process and may affect receptor desensitization.

Desensitization is also a general property of other receptor families such as receptor tyrosine kinases and ligand-gated ion channels. For example, receptor tyrosine kinases undergo agonist-induced dimerization and autophosphorylation that initiates receptor internalization (see Receptor Internalization). Phosphorylation of serine and tyrosine residues may also modulate the desensitization of ion channels such as the nicotinic acetylcholine receptor at the neuromuscular junction. Nevertheless, the molecular mechanism that mediates channel opening by agonist binding, and then allows the channel to close (desensitize) even though agonist remains bound, is not understood in detail.

It should be noted that fast inactivation of receptor signaling not only involves the desensitization of the receptor but also the components of the downstream signaling cascade. The deactivation of active $G\alpha$ subunits is controlled by the intramolecular hydrolysis of bound GTP, allowing it to reform the inactive heterotrimer. Termination of G protein-mediated signaling in vivo is 10- to 100-fold faster than the in vitro rate of GTP hydrolysis by $G\alpha$ subunits, suggesting

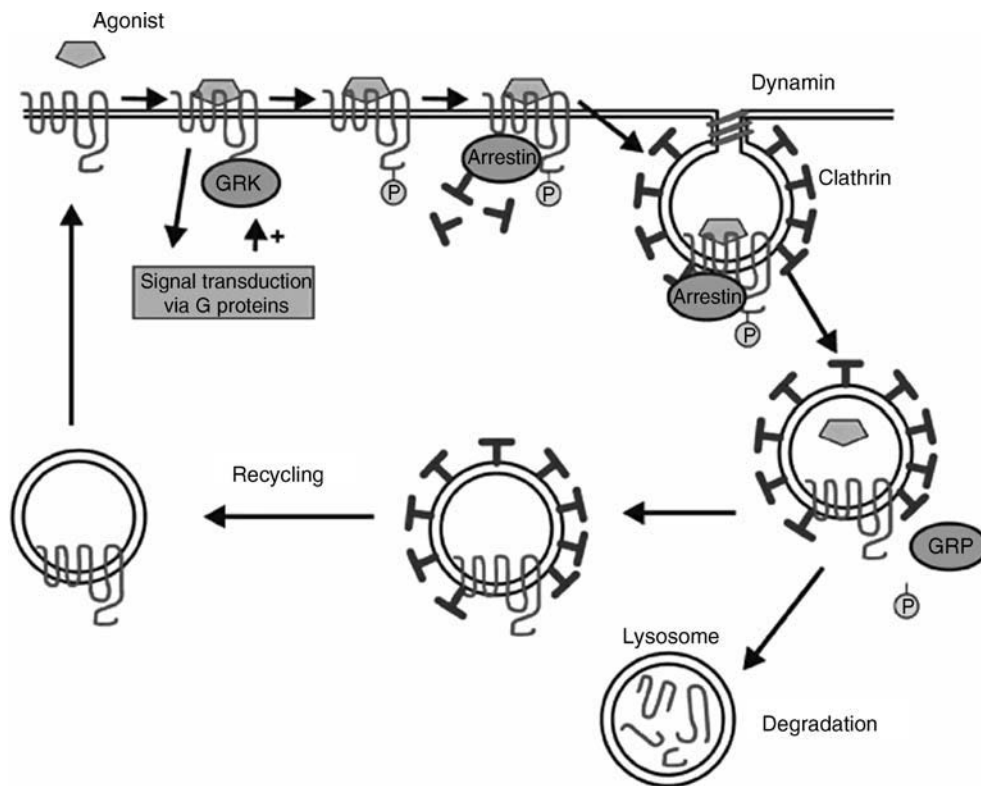
the existence of GTPase-activating proteins (GAPs). Indeed, so-called “regulators of G protein signaling” (RGS) have been identified as potent GAPs for $G\alpha$ subunits which are required to achieve timely deactivation.

Receptor Internalization

Agonist-induced receptor **internalization** (also called receptor endocytosis or sequestration) has been observed for many membranous receptors including GPCRs. Following GRK-mediated receptor phosphorylation, arrestins bind to the receptor and recruit other proteins such as AP-2, dynamin, and clathrin. Then, clathrin-coated pits are formed and the receptors internalize. Subsequently, endosomal acidification permits dissociation of the ligand and dephosphorylation of the receptor by cytosolic phosphatases (Fig. 1). Internalized receptors can recycle to the cell surface or can be degraded in lysosomes, a process known as receptor downregulation (see Receptor Down-Regulation). The predominant pathway for internalization requires a concerted action

of dynamin, arrestin, and clathrin. But there are mechanistic differences in arrestin-mediated receptor trafficking. So-called “class A” receptors (e.g., β_2 -adrenergic receptor) bind to β -arrestin transiently, traffic with it to clathrin-coated vesicles, then dissociate and rapidly recycle. “Class B” receptors (e.g., V_2 vasopressin receptor) bind β -arrestin more tightly and internalize together with it. It should be noted that there are additional pathways of GPCR endocytosis. Internalization of the M_2 muscarinic acetylcholine receptor also requires dynamin, but proceeds in an apparent β -arrestin- and clathrin-independent manner.

In some cases, receptor inactivation, e.g., of the V_2 vasopressin receptor, is mediated by agonist-induced enzymatic cleavage of the GPCR. This nonendocytic proteolysis is promoted by a plasma membrane-associated metalloprotease. Proteinase-activated receptors (PARs) such as the thrombin receptor also follow a distinctly different pathway. PARs require the enzymatic cleavage of their N terminus, and the newly generated N terminus activates the receptor. Once



Tolerance and Desensitization. Figure 1 Agonist-induced internalization of GPCRs (G protein-coupled receptors). The model depicts the agonist-induced regulation of GPCR phosphorylation, internalization, recycling, and degradation. Agonist activation of many GPCRs results in receptor phosphorylation by GPCR-specific kinases (GRK). The phosphorylated GPCR recruits β -arrestin, which initiates receptor targeting to clathrin-coated pits. Endosomal acidification permits dissociation of the ligand. The GPCR is dephosphorylated by a G protein-coupled receptor-specific phosphatase (GRP). Internalized receptors can recycle to the cell surface or are degraded in lysosomes.

activated, PARs are internalized into endosomes and sorted to lysosomes for degradation. Recovery of functional receptors at the plasma membrane requires synthesis of new receptors or mobilization of intact receptors from intracellular pools.

Receptor tyrosine kinases, such as the epidermal-growth factor (EGF) receptor, also undergo agonist-induced endocytosis which is assumed to follow a two-step process. First, the agonist induces receptor dimerization and conformational changes that allow for cross-phosphorylation of tyrosine residues. In a second step, the phosphorylated receptor tyrosine kinase recruits adaptor proteins such as Cbl protein and endophilin, which promote receptor internalization by forming clathrin-coated pits. Receptor activation also induces ubiquitination, a sorting signal for receptor degradation in lysosomes. Interestingly, GPCRs can cross-desensitize receptor tyrosine kinases through clathrin-mediated endocytosis.

Ligand-gated ion channels, such as GABA type A receptor, can also undergo clustering and internalization that is evoked when receptors are occupied by agonists. Internalized receptors can be rapidly recycled to the cell surface, a process that is regulated by PKC. Similar to GPCRs, a portion of the intracellular GABA type A receptors derived from ligand-dependent endocytosis is targeted to degradation pathways.

Receptor Down-Regulation

The decrease in the total cellular receptor number is a process known as receptor downregulation. It is caused by chronic agonist exposure and occurs in hours or days. This process is mainly agonist-mediated but there is also evidence for agonist-independent pathways of cell surface receptor depletion. Recovery from receptor downregulation is reversible but slow. One mechanism of receptor downregulation involves lysosomal targeting and the accelerated degradation of internalized receptors (Fig. 1). The factors that determine the fate of an internalized receptor (recycling pathway vs. degradation) are currently under investigation. Conjugation of proteins with ubiquitin, an abundant intracellular protein, is a signal for the rapid degradation of many cytosolic and membranous proteins. It has been demonstrated that receptor tyrosine kinases and GPCRs undergo agonist-induced ubiquitination. There is evidence that β -arrestins act as obligate adaptors to guide ubiquitin ligases to the receptor. Furthermore, GPCR activation can provoke sustained β -arrestins ubiquitination.

The regulation of receptor synthesis is a second component of receptor downregulation. It involves processes that reduce gene transcription, mRNA stability, and receptor half-life time. It should be noted that mechanisms in addition to the regulation of the receptor number may account for tolerance development. Second messenger levels and enzyme activities that participate in the signaling of a given receptor are

found to be up- or downregulated to compensate for chronic over stimulation of a distinct signal transduction cascade.

Pharmacological Relevance

Desensitization, internalization and downregulation of receptors are mechanisms that eventually lead to a loss of agonist efficacy. These processes together with agonist removal have been implicated in **tachyphylaxis** and in the development of tolerance and drug **dependence**. Vice versa, chronic administration of antagonists and inverse agonists can increase the number of receptors and may account for super sensitivity seen, e.g., after rapid termination of a long-term treatment with β -adrenergic receptor blockers. Understanding the molecular mechanisms of long-term receptor expression regulation may help to influence or even prevent therapeutically undesired effects.

Many mechanistic questions are still puzzling, but recent studies with genetically engineered mice have provided some new insights in the processes of desensitization and tolerance development. The desensitization of the μ -opioid receptor and development of antinociceptive tolerance was abolished in mice lacking β -arrestin-2. As a result of gene deletion, these mice showed a remarkable potentiation and prolongation of the analgesic effect of morphine. Interestingly, the deletion of β -arrestin-2 does not prevent the chronic morphine-induced upregulation of adenylyl cyclase activity, a cellular marker of dependence, and the mutant mice still became physically dependent on the drug. In concert with this finding, several studies indicate that highly addictive opiate drugs such as morphine have a reduced ability to induce the desensitization and endocytosis of opioid receptors. Therefore, the development of physical dependence and addiction appears to be independent from desensitization and endocytosis of opioid receptors. Additional mechanisms of tolerance formation are discussed. These include upregulation of adenylyl cyclase expression and activity, modulation of protein kinase activity as well as upregulation of members of the RGS family.

Agonist-dependent and -independent changes in receptor density can contribute to pathological situations. There is a downregulation of cardiac β -adrenergic receptors in dilated cardiomyopathy, probably as a consequence of increased sympathetic tone. A rapid upregulation of β -adrenergic receptors is characteristic of myocardial ischemia and hyperthyreosis. Blockade of β -adrenergic receptors inhibits the basal receptor activity and action of endogenous catecholamine and may explain part of the beneficial effects of β -blockers in both diseases.

Taken together, the regulation of time course and extent of receptor desensitization and tolerance development involves complex cellular processes. Detailed understanding of the molecular mechanisms of receptor

inactivation may improve the chronic drug treatment of patients and may offer new targets of therapeutic interventions.

- Drug Addiction/Dependence
- Drug–Receptor Interaction
- G-protein-coupled Receptors

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Toll-like Receptors

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Definition

Toll-like Receptors (►TLRs) are type-1 integral membrane glycoproteins with molecular weights of 91–115 kDa. They consist of a Toll/IL-1 Receptor (TIR) domain in their C-terminii and a set of Leucine rich repeats (►LRRs) in their N-terminii [1]. They are critical receptors in the ►innate immune system—the body's first defence against pathogens, due to their ability to recognise microbial products and initiate an internal signal cascade to alert the body to attack [1]. To date 10 human TLRs have been discovered each with the ability to recognise a different microbial product.

Basic Mechanisms Innate Immune System

The innate immune system is found across all organisms and is thought to have originated before the split into animal and plant kingdoms. It is the body's primary response to attack. Receptors used in this system must be capable of recognising a broad range of pathogens. This

works by the recognition of molecules common to the pathogens that are absent in the host. These molecules are recognised by the TLRs. Other important receptors in innate immunity include the secreted mannan-binding lectins and the cytoplasmic NOD like receptors (NLRs).

Discovery

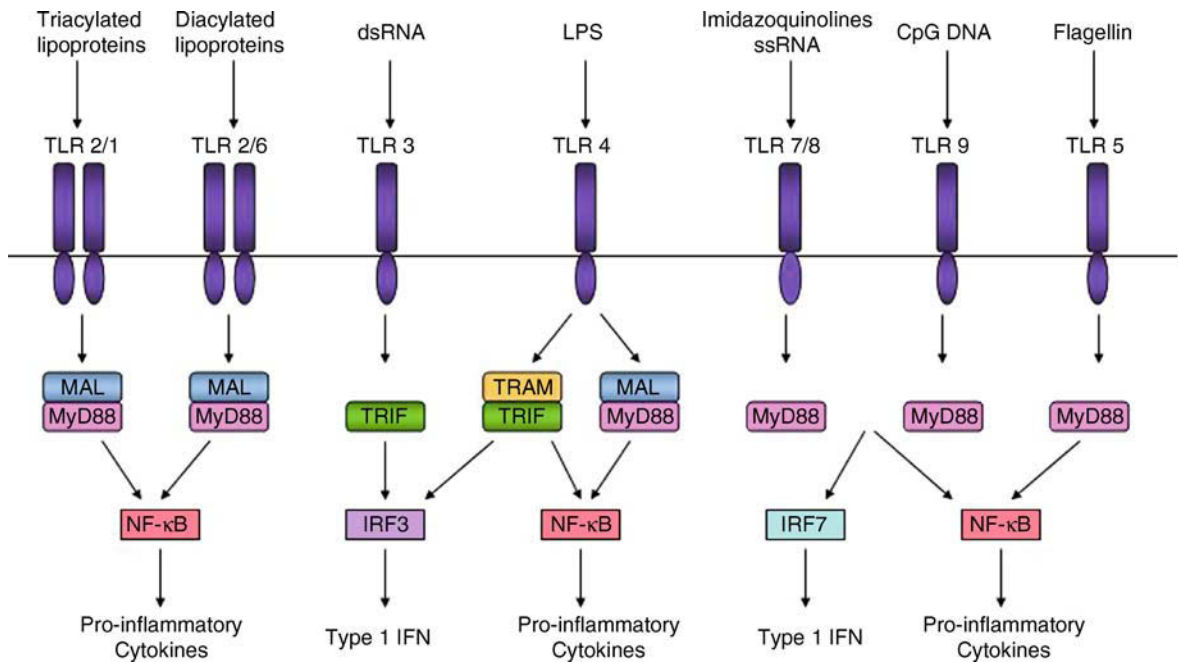
The discovery of TLRs can be traced back to the investigations into the genetics of the fruit fly *Drosophila melanogaster* in the mid-1980s. The Toll receptor in *Drosophila* was shown to be crucial in the establishment of the dorso-ventral pattern during development in flies. It was not until almost 10 years later that it was found that Toll was also important in anti-fungal host defence. Toll consists of an N-terminal leucine rich repeat (LRR) motif and a C-terminal domain highly homologous to the cytosolic domain of the type I IL-1 receptor (IL-1RI) and hence was named the Toll/IL-1R (►TIR) domain. The homology to IL-1RI indicated that Toll would have a role in host defence, similar to IL-1.

The hunt then began for a mammalian homologue to Toll and in 1997 the first protein similar to Toll was discovered. This protein activated the transcription factor NF- κ B when over expressed. Five human Tolls were then reported and named Toll-like receptors (TLRs). One of these, TLR4, was identical to the first Toll discovered in mammals. Importantly it was then demonstrated that in mice resistant to the Gram negative protein lipopolysaccharide (LPS), termed C3H/HeJ mice, there was a mutation in the *TLR4* gene identifying TLR-4 as the receptor for LPS. Subsequently other TLRs were shown to recognise various microbial products.

What TLRs Recognise

TLRs are transmembrane proteins found on the plasma membrane and on endosomal membranes. The ability of the TLRs to recognise microbial products comes from the 19–25 copies of the LRR motif. The differences in these LRRs are what give the TLRs the ability to bind different components of pathogens.

TLR-1, 2, 4, 5, 6 and 10 are found on the plasma membrane and TLR-7, 8 and 9 are found on the endosomal membranes. TLR-3 has been seen in both depending on the cell line examined. The ligands recognised by the TLRs are outlined in Fig. 1. TLR-2 forms dimers with TLR-1 or 6 to recognise triacylated and diacylated lipopeptides, respectively. All other TLRs probably form homodimers. TLR-4 binds LPS and TLR-5 binds a protein on the flagella of bacteria called flagellin. TLR-3, 7 and 8 are involved in recognition of viral nucleic acids, with TLR-3 recognising double-stranded RNA, and TLR-7 and 8 recognising single-stranded RNA. TLR-7 also recognises the synthetic viral compound imiquimod. TLR-9 is involved in the recognition of unmethylated CpG motifs of pathogens. TLR-10 is found on the same locus as TLR-1



Toll-like Receptors. Figure 1 Overview of TLR signalling pathways. TLRs detect a broad range of conserved microbial structures thereby initiating an immune response against the invading pathogen. Upon activation, the adapter proteins Mal, MyD88, TRIF and TRAM are differentially recruited to the various receptors. MyD88 is utilised by all the TLRs with the exception of TLR3. TRIF is activated downstream of TLR3 and TLR4. Mal and TRAM act as bridging proteins, recruiting MyD88 and TRIF, respectively to TLR4. The recruitment of the adapter proteins leads to the activation of signalling pathways, which culminate in the activation of specific transcription factors, such as NFκB and IRFs. This subsequently leads to the production of proinflammatory cytokines and/or Type 1 IFNs.

and 6 and may in fact interact with them or TLR-2 to signal. At present its ligand remains unclear [1].

Once the TLRs have bound their respective ligand they initiate a signalling cascade to alert the host to the presence of a threat. This signal begins with specific adapter proteins and leads to the activation of transcription factors such as NFκB, IRF-3 and IRF-7 as shown in Fig. 1. These activated transcription factors cause changes in gene expression typically leading to the production of ►cytokines.

Activation of the Signalling Cascade

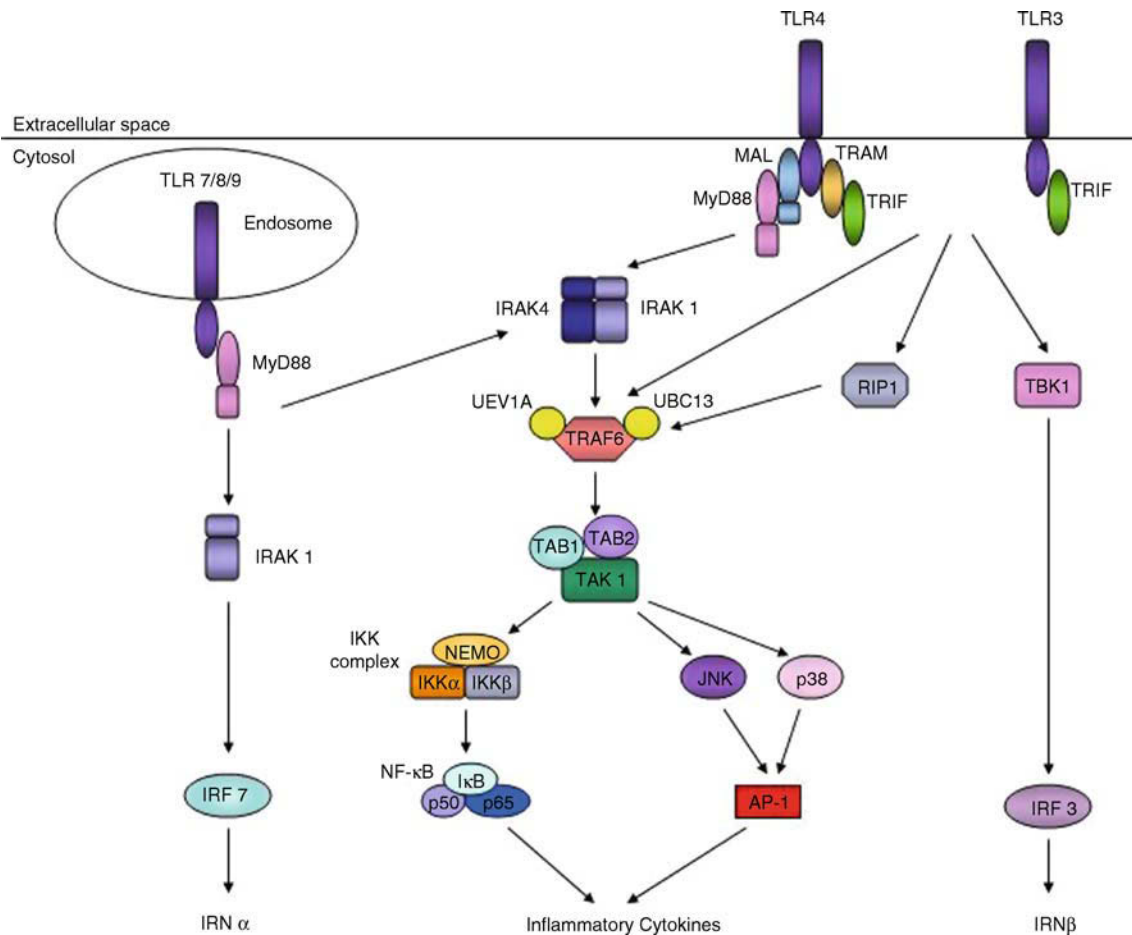
The function of the TIR domain found on each TLR is to allow adapter proteins to bind the TLRs. These adapter proteins then become somehow activated, presumably by conformational changes, and bind other proteins in the signalling pathway. To date five adapter proteins have been discovered four of which aid the signal pathway: Myeloid Differentiation factor 88 (MyD88), MyD88 adapter like protein (Mal), TIR domain containing adapter inducing IFN-β (TRIF) and TRIF-related adapter molecule (TRAM) and one that is inhibitory: SAM and ARM containing protein (SARM) [2]. All the adapter proteins contain a TIR domain allowing them to bind the TIR domain of the activated TLRs.

The MyD88 Dependent Response

MyD88 was the first adapter protein to be discovered. As well as the TIR domain it contains a death domain (DD). MyD88 is used by all TLRs to signal with the exception of TLR-3, which requires TRIF. Its signalling pathway is outlined in Fig. 2.

To begin the signalling cascade MyD88 binds to the TIR domain of an activated TLR through its own TIR domain. This binding leads to the recruitment of IL-1 receptor associated kinase-4 (►IRAK-4) through its death domain. IRAK-1 is also recruited to MyD88 through its interaction with Toll-interacting protein (Tollip).

Hyperphosphorylation of IRAK-1 by itself and IRAK-4 causes IRAK-1 to dissociate from the membrane-bound complex. Tumour necrosis factor (TNF) receptor-associated factor-6 (►TRAF-6), a cytoplasmic protein, is activated by IRAK-1 and with TAB-2, another cytoplasmic protein, activates transforming growth factor-β (TFG-β)-activating kinase (TAK-1). During this process both TRAF-6 and TAK-1 become ubiquitinated. TAK-1 then promotes activation of the IκB kinases, or the ►IKK family, IKKα and IKKβ (found in a complex with NFκB-essential modulator [NEMO]), which phosphorylate the IκB family, notably IκB-α. IκB-α is an inhibitor of NFκB as it sequesters NFκB in an



Toll-like Receptors. Figure 2 MyD88 and TRIF dependent pathways activated by the TLRs. Upon ligand binding, MyD88 is recruited to the receptor and recruits IRAK-4. IRAK-1 is subsequently recruited and activated by IRAK-4. IRAK-1 is responsible for the activation of TRAF-6, which with TAB-2 activates the kinase TAK-1. Substrates for TAK-1 include the IKK complex, JNK and p38. IKK phosphorylates IκB-α, which induces its ubiquitination and degradation. This allows NFκB to translocate to the nucleus and induce gene expression. JNK and p38 mediate the activation of AP-1. Both these transcription factors act in concert to produce inflammatory cytokines. Downstream of TLR-7, 8 and 9, MyD88 is also responsible for the activation of IRF7. The TRIF dependent pathway results mainly in the activation of IRF-3 via TBK-1. IRF-3 is involved in the production of type 1 IFNs. TRIF can also mediate the activation of NFκB via RIP-1 and/or TRAF-6.

inactive form in the cytosol. When it is phosphorylated it is marked for polyubiquitination and ultimately degradation by the 26 S proteasome. This allows NFκB to translocate to the nucleus and bind transcription start sites on target genes. These genes code for proteins involved in immunity and inflammation.

TAK-1 can also activate c-jun N-terminal kinase (▶JNK) and p38 both of which are MAP kinases. JNK activates the transcription factor AP-1 and p38 is involved in mRNA stabilisation. This pathway is also capable of activating IRF-7 in dendritic cells in response to TLR-7 and TLR-9 ligands.

Mal

Mal was the second adapter to be discovered. It is a bridging adapter connecting MyD88 to TLR-4 and

TLR-2. Evidence for the bridging role of Mal comes from the presence of a phosphatidylinositol 4,5-bisphosphate (PIP2) binding domain at its N-terminus. This domain recruits Mal to areas of the plasma membrane rich in PIP2 and these areas have been shown to contain TLR-4.

There is evidence for another role for Mal besides recruiting MyD88. This is due to the discovery of several other domains in the protein. These include a TRAF-6 binding domain allowing Mal to activate TRAF-6 without MyD88, a PEST domain commonly seen in proteins targeted for ubiquitination and a caspase-1 cleavage site that cleaves Mal into two proteins one of 4 kDa and one of 21 kDa. Mal is also tyrosine phosphorylated by Bruton's tyrosine kinase (Btk) and this phosphorylation targets it for degradation by SOCS-1.

The TRIF Dependent Response

TRIF was the third adapter discovered and is utilised by TLR-3 and TLR-4. It contains a TIR domain and a TRAF-6 binding domain. TLR-3 signalling is entirely through TRIF but in TLR-4 signalling TRAM is also required. An outline of the TRIF pathway is shown in [Fig. 2](#).

To initiate TLR-3 signalling TRIF binds the TIR domain of TLR-3 through its own TIR domain and three signalling pathways are activated. In the first pathway TRIF can recruit TRAF-6 via its TRAF-6 recruitment domain and initiate the same series of events seen in the MyD88 dependent pathway. However, the role of TRAF-6 is still unclear due to different dependencies on it in various cell types. Murine macrophages deficient in TRAF-6 respond normally to TLR-3 ligands but murine embryonic fibroblasts (MEFs) deficient in TRAF-6 do not. This area of TRIF signalling requires further investigation.

TRIF can also activate IRF-3 leading to the production of type I interferons (IFNs).

This second pathway begins with the direct binding of TRIF to TLR-3 and the activation by it of two kinases: inducible IKK (IKK- ϵ also known as IKK- β) and TRAF family member-associated NF κ B activator (TANK)-binding kinase-1 (TBK-1). The activation of these two kinases leads to the phosphorylation of IRF-3 and IRF-7 allowing them to enter the nucleus and induce the expression of IFN- α (IRF-7) and IFN- β (IRF-3 & 7). It has been shown that TBK-1 binds to the N-terminus of TRIF in the same area as TRAF-6 suggesting that in a TLR-3 response either TBK-1 or TRAF-6 bind TLR-3, but never both.

The third signalling pathway of TRIF utilises its C-terminus. This region contains a RIP homology interaction motif (RHIM) that binds RIP-1 and RIP-3. RIP-1 phosphorylates IKK- β and the NF κ B pathway can be activated. RIP-3 is a negative regulator of TRIF competing for RIP-1 [2].

TRAM

TRAM was the fourth adapter discovered and has only been seen to have a role in TLR-4 signalling. It contains a TIR domain and a myristoylation site. When TRAM is myristoylated it becomes bound to the plasma membrane and can bind to TLR-4 through its TIR domain. TRAM then allows TRIF to bind it and activate the pathways associated with TRIF as outlined above for TLR-3.

TRAM is subject to control through phosphorylation by protein kinase C- ϵ . It is phosphorylated on serine 16 which is located close to the myristoylation site which is TRAM cannot signal without this phosphorylation or if the myristoylation site has been mutated.

SARM

SARM was the final adapter to be identified. It contains a TIR domain, two sterile- α (SAM) motifs adjacent to

the TIR domain and HEAT/Armadillo repeats. SARM is a negative regulator of NF κ B and IRF activation in the TRIF pathway alone. Experiments show that the presence of SARM can block the TRIF-dependent pathway while the MyD88 dependent pathway remains functional. It has also been shown that the C-terminus of SARM is sufficient to cause this negative regulation. It is still unclear how SARM inhibits the TRIF pathway.

Cytokine Production

The key end result of TLR signalling is the induction of cytokines. Cytokines are proteins produced during an immune response that allow the maturation, activation and differentiation of effector cells in the immune system. The activation of NF κ B and AP-1 by the MyD88 and the TRIF dependent pathways leads to the production of proinflammatory cytokines such as IL-6, TNF- α and various chemokines. This pathway can also activate IRF-7 via TLR-7 and TLR-9 allowing Type-I interferons to be produced.

The activation of IRF-3 through the TRIF dependent pathway allows for chemokines such as RANTES to be produced. It also leads to the production of IFN- α and IFN- β , which are involved in anti-viral immunity. The TRIF pathway, activated by either TLR-3 or TLR-4, can also induce MHC class-II expression and co-stimulatory molecules, thus leading to T-cell activation. This provides an important link between innate and adaptive immunity.

Pharmacological Intervention

Though TLRs are central to protecting the host they are also linked with disease. They can become over activated during infection, or by the presence of endogenous ligands released from damaged cells and cause inflammation. Examples of the diseases associated with TLRs are shown in [Table 1](#). Several TLRs have been specifically seen to be activated in infectious and inflammatory diseases. The knowledge that TLRs could be involved in disease opened the area of TLR research into drug targeting to counteract the negative effect of TLRs on the host. In addition activation of TLRs will stimulate the immune response and this has potential applications in adjuvants for vaccines, promotion of anti-viral host defence, or the enhancement of anti-tumour immunity. An outline of the compounds that target TLRs can be found in [Table 2](#) [3].

TLR-4

Several TLR-4 adjuvants for vaccines have been developed to date. An example of these is monophosphoryl lipid A (MPL) a modified version of lipid A found in LPS [4]. It has been used extensively in clinical trials as it is far less toxic than LPS. It is hoped to use MPL in vaccines against infectious diseases, allergies and cancer. Derivatives of MPL have now been

Toll-like Receptors. Table 1 Disease Associations of TLRs

TLR	Associated Diseases
TLR4	EAE, Atherosclerosis, Sepsis, Asthma, COPD, RSV bronchitis, Arthritis
TLR2	Candidiasis, Asthma, Arthritis
TLR3	Arthritis, Lethal encephalitis
TLR5	Legionnaire's disease
TLR7/8	SLE, Scleroderma, Sjögren's syndrome
TLR9	SLE, Malaria
TLR 2,3,4,9	Diabetes, Cardiomyopathy

COPD, chronic obstructive pulmonary disease; EAE, experimental autoimmune encephalomyelitis; RSV, respiratory syncytial virus; SLE, systemic lupus erythematosus.

Toll-like Receptors. Table 2 Therapeutic Targeting of TLRs

TLR	Compound	Action	Indication	Status
TLR4	Supervax™	Agonist	Hepatitis B	Approved (EU)
	Fendrix®	Agonist	Hepatitis B	Approved (Argentina)
	E6020	Agonist	General vaccine adjuvant	Preclinical
	Cervarix®	Agonist	Human papillomavirus	Pre-approval
	Pollinex®Quattro	Agonist	Allergic rhinitis	Marketed (EU)
	Ragweed SC/Pollinex®Quatro	Agonist	Allergic rhinitis (Ragweed)	Phase II clinical trials
	Stimuvax®/BLP25	Agonist	Non-small cell lung cancer	Phase II clinical trials
	TAK-242	Antagonist	Severe sepsis	Phase III clinical trials
	Eritoran	Antagonist	Severe sepsis	Phase III clinical trials
TLR7	Aldara™	Agonist	Basal cell carcinoma Genital warts	Approved
	852A	Agonist	Melanoma	Phase II clinical trials
	ANA975	Agonist	Hepatitis C	Phase I clinical trials
	Resiquimod (R-848)	Agonist	Genital Herpes	Phase II clinical trials discontinued
TLR7/9	IRS 954	Antagonist	Lupus	Preclinical
TLR9	CpG-ODN 7909	Agonist	Melanoma	Phase I clinical trials
	PF3512676	Agonist	Non-small cell lung cancer	Phase III clinical trials
	1808 ISS	Agonist	Colorectal cancer	Phase I clinical trials
	1808 ISS	Agonist	Asthma	Phase II clinical trials
	IMOxine	Agonist	Renal cell carcinoma	Phase II clinical trials
	Second generation CpG-ODN	Agonist	Asthma	Preclinical
	HYB2093 (CpG-ODN)	Agonist	Asthma	Preclinical

produced and have been shown to be effective in vaccines against hepatitis B and human papilloma virus.

TLR-4 has also been targeted in defence against allergy. These compounds also use MPL linked to antigens to prevent allergic reaction specifically against ragweed for example. Anti-tumour peptide vaccines have also been developed again using MPL or its derivatives.

Antagonists of TLR-4 have been developed to prevent an excessive reaction to infection in the body. TAK-242 and Eritoran are both in phase III trials to help combat

severe sepsis due to Gram negative bacteria. Both are lipid A analogues and prevent LPS binding to TLR-4.

TLR-7/8

TLR-7 and 8 have also been investigated as a way of enhancing the body's response to viral attack. This has been used to target cancer and infectious diseases. Treatment of basal cell carcinoma using an imiquimod cream has been approved and meloma targeting drugs are in phase II trials. Imiquimod is also approved for use against papilloma-induced genital warts [4].

In infectious disease compounds such as ANA975 (phase I trials) and Resiquimod (phase II trials) have been developed to target Hepatitis C and genital herpes, respectively. IRS954 is a TLR-7 and 9 antagonist in pre-clinical trials for Systemic Lupus Erythematosus (SLE).

TLR-9

CpG-ODN7907 is a TLR-9 agonist, which has been developed to target melanoma by attaching it to the melan-A peptide and is in phase I clinical trials. Other examples include PF3512676 used in combination with chemotherapy to treat non-small cell lung cancer, which is in phase III trials. 1018 ISS is a TLR-9 agonist being tested in combination with Rituxan to treat non-Hodgkins lymphoma [3].

TLR-9 has also been used to target asthma with several compounds in preclinical trials such as second generation CpG-ODNs and HYB2093. 1018 ISS has also been tested in asthma. Defence against infectious disease is also enhanced through TLR-9. CpG10101 was in phase II trials as a Hepatitis C target but has been discontinued.

These compounds represent only some of the efforts being made to target the TLRs and their signalling pathways. Targeting TLRs holds great promise in the effort to find new therapies for infectious, allergic and inflammatory diseases, and cancer.

- Cytokines
- DNA Vaccination and Genetic Vaccination
- Immune Defense
- Genetic Vaccination
- Table appendix: Receptor Proteins

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Tonic-clonic Convulsions

Tonic-clonic convulsions are abnormal motor behavior during a seizure characterized by slow movements with high muscle tension (tonic phase) and subsequent repetitive oscillating movements of limbs (clonic phase).

- Antiepileptic Drugs

Topoisomerase

Topoisomerase enzymes control and modify the topologic states of DNA. The mechanisms of these enzymes involve DNA cleavage and strand passage through the break, followed by religation of the cleaved DNA. Two main forms of topoisomerase exist. The type I topoisomerase of mammals is a 100 kD monomeric protein whose activity is ATP-independent. This enzyme binds to double-stranded DNA and cleaves one of the DNA strands of the duplex, simultaneously forming an enzyme-DNA covalent bond between a tyrosine residue and the 3'-phosphate of the cleaved DNA. The type II topoisomerases are dimeric enzymes, which are ATP-dependant. Two isoforms of topoisomerase II exist, topoisomerase α and β , with apparent molecular weights of 170 and 180 kD. Topoisomerase II cleaves the two complementary strands of DNA four base pairs apart and the resulting 5'-phosphoryl groups become covalently linked to a pair of tyrosine groups, one in each half of the dimeric topoisomerase II enzyme. Several groups of drugs are known that selectively inhibit topoisomerases in bacteria (quinolones) or mammalian cells (etoposide, tenoposide). Quinolones are used to treat bacterial infections; inhibitors of mammalian topoisomerases are cytostatic drugs used for the treatment of cancer.

- Quinolones
- Antineoplastic Agents

Topotecan

- Camptothecins

TOR Signalling

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Synonyms

Target of Rapamycin encoded by: *TOR1* and *TOR2* in yeast; *mTOR*, replaces *FRAP*, *RAFT*, *RAPT* or *SEP* in mammals

Definition

Very large Serine/Threonine kinases and the molecular Target of Rapamycin, a naturally occurring secondary metabolite, TOR proteins function within multiprotein complexes to couple cell growth and stress responses to environmental and developmental cues.

Basic Mechanisms

TOR genes were originally described genetically in *Saccharomyces cerevisiae* as the targets of the antifungal and immunosuppressive compound, rapamycin. Subsequent to their discovery in yeast, TOR genes have been found in all eukaryote genomes examined. Eukaryote TOR proteins share 40–60% identity and belong to a group of kinases known as phosphatidylinositol kinase-related and kinases (PIKK)s. PIKKs are very large (~280 kDa) have a characteristic domain structure as diagrammed for TOR in Fig. 1.

Rapamycin is a macrocyclic lactone produced by *Streptomyces hygroscopicus*. This bacterium was originally cultured from a soil sample collected on Easter Island (known locally as Rapa Nui; hence the name rapamycin). Parenthetically, rapamycin shares an interesting mode of action with two other antifungal and immunosuppressive compounds, ▶FK506 and ▶cyclosporin A. Inside cells, rapamycin first binds to FKBP12, a small protein receptor known as an ▶immunophilin. FKBP12 is not an essential protein but is an important cofactor required for rapamycin to bind and inhibit TOR.

Two TOR Complexes

Biochemical purification of TOR demonstrated that this protein functions as the catalytic component of two distinct multiprotein complexes known as TOR complex 1 (TORC1) and TOR complex 2 (TORC2). Like TOR, these complexes appear to have been structurally and functionally conserved from yeast to man. The mammalian equivalents are known as mTORC1 and mTORC2.



TOR Signalling. Figure 1 Domain structure of TOR and related PIKKs. HEAT: a domain first identified in huntingtin, elongation factor 3, the PR65/A subunit of protein phosphatase 2A (PP2A), and TOR; thought to mediate protein–protein interactions. FAT: a modified HEAT repeat found in FRAP, ATM, and TRRAP. FRB: where FKBP12–rapamycin binds, a domain specific to TOR. Kinase: although it resembles a lipid kinase, this domain phosphorylates serine and threonine residues. FATC: a small, essential domain found at the carboxy-terminus. This domain is always found in conjunction with the FAT domain suggesting that these two domains interact.

As illustrated in Fig. 2, these complexes are likely dimeric. Curiously, only when in TORC1 is TOR/mTOR bound and inhibited by the rapamycin–FKBP12 complex. Rapamycin has been an incredibly important tool to dissect the molecular function of TORC1/mTORC1 and as outlined below, rapamycin and related derivatives have ever-increasing clinical potential. No small molecule inhibitor of TORC2/mTORC2 has been identified and consequently understood the functions of this complex is relatively less well developed.

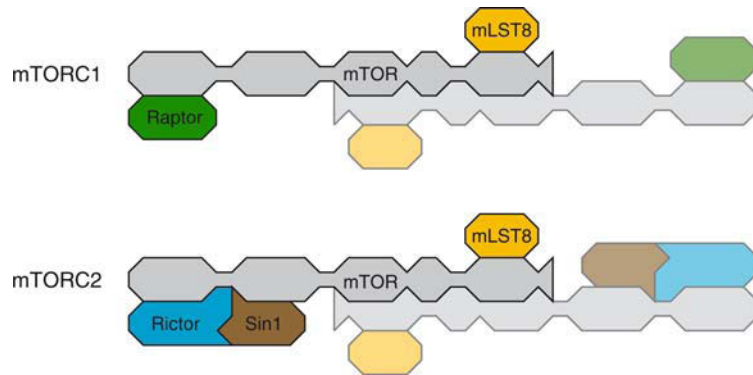
Physiological Roles of TORC1 and TORC2

Studies in yeasts (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*), slime mould (*Dictyostelium discoideum*), worm (*Caenorhabditis elegans*), fly (*Drosophila melanogaster*) and mammalian systems have all contributed to our understanding of TOR signalling.

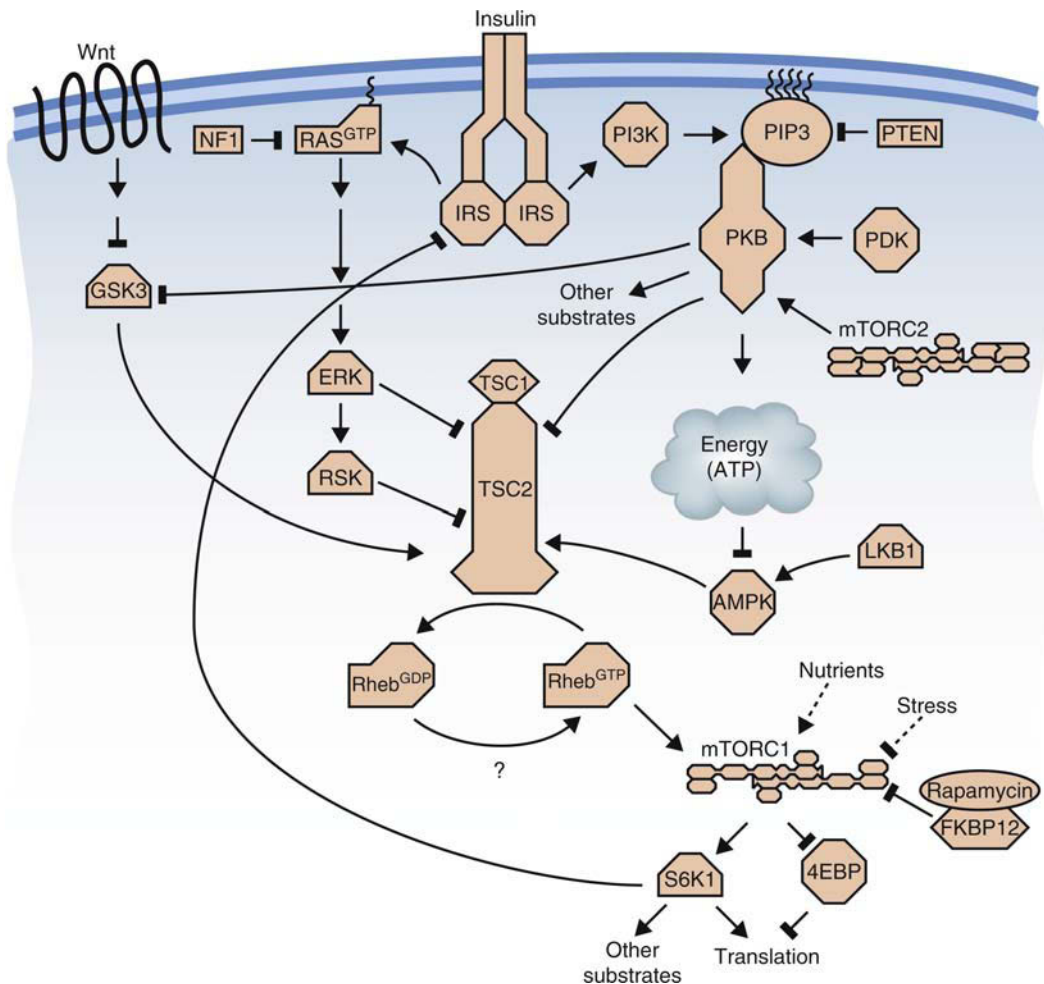
TORC1 Regulates Cell Growth and Stress Responses

The processes regulated by TORC1 are still best defined in budding yeast. Studies with rapamycin have demonstrated that TORC1 controls cell ▶growth, or said more precisely, the accumulation of mass, in a number of ways. For example, TORC1 enhances translation initiation, expression and assembly of the protein translation machinery, mRNA stability and the activities of permeases that import amino acids. TORC1 additionally blocks turnover of existing mass by inhibiting a nutrient recycling programme known as ▶macroautophagy. Although the mechanisms by which TORC1 regulates these processes are only beginning to be defined, many of these rapamycin-sensitive readouts are conserved in mammals. Indeed, mTORC1 regulates translation initiation via its two well-documented substrates, 4E-BP1, a negative regulator of translation initiation that is inhibited by mTORC1, and S6K1, a positive regulator of translation initiation that is activated by mTORC1 (Fig. 3). TORC1 in yeast is essential for viability. Similarly, exposure of early mouse embryos to rapamycin also arrests cell proliferation indicating that mTORC1 performs an essential function at this point of development. In metazoans, most “growth” takes place during development; adults in contrast, have largely stopped growing. Consistently, although certain cell types still respond, systemic rapamycin treatment is generally well tolerated in adults (see below).

Yeast cells exposed to noxious stress arrest their growth and increase expression of ▶stress response genes. Thought of another way, “growth” and “stress response” appear to be mutually exclusive states. Consistently, TORC1 also promotes growth by antagonizing the nuclear accumulation and activity of a number of stress-activated transcription factors. Indeed, ▶transcriptome profiling in yeast indicated that



TOR Signalling. Figure 2 Dimeric structure of mTORC1 and mTORC2. mTORC1 contains mTOR, raptor and mLST8. Raptor binds the HEAT repeats of mTOR, mLST8 binds the kinase domain of mTOR. mTORC2 contains mTOR, rictor, Sin1 and mLST8. Rictor and Sin1 cooperatively bind the HEAT repeats of mTOR.



TOR Signalling. Figure 3 Simplified schematic of the mTOR signalling network. Arrows and bars indicate positive and negative signals respectively. Dashed lines indicate that signalling details are not understood. PKB and S6K1 have many more substrates than indicated. See text and [Table 1](#) for details.

rapamycin treatment, nutrient withdrawal, and application of noxious stress all elicit very similar transcriptional programmes. Based on such correlative observations it is hypothesized that nutrient abundance is a physiological activator of TORC1 while noxious stress is a physiological inhibitor of TORC1, however, detailed signalling mechanisms are not known. In contrast to unicellular organisms, metazoans must coordinate cell growth with both organ and organismal growth and this is accomplished via circulating growth factors. As described below, recent studies have elucidated much of the signalling pathways that couple growth factor receptors to mTORC1.

The Physiological Roles of TORC2 are Still Being Elucidated

Since no rapamycin-equivalent exists with which to inhibit TORC2, the functional interrogation of this complex has relied mainly on mutational studies. In *S. cerevisiae*, characterization of cells possessing diminished TORC2 activity demonstrated that TORC2 regulates endocytosis, [▶actin polymerization](#) and consequently cell polarity. Recent studies in the slime mould *Dictyostelium discoideum* also support a role for TORC2 in regulating cell polarity, but curiously, studies in the fission yeast *S. pombe* have not established a link between TORC2 and actin/cell polarity but rather suggest that TORC2 in this organism is required for proper stress responses. mTORC2 probably regulates both cell polarity and stress response via its direct pleiotrophic substrate PKB/akt ([Fig. 3](#)).

Like TORC1, TORC2 is also essential for viability in yeast. Constantly, Homozygous ablation of mTORC2-specific components (e.g., *riCTOR*^{-/-} and *sin1*^{-/-}) results in early embryonic lethality indicating that mTORC2 also performs an essential function at this point of development. The functional requirement of mTORC2 in adults has not yet been evaluated and in no organism are the upstream regulators of TORC2 known.

[▶Growth factor](#) and hormone signalling cascades link mTORC1 dysregulation to cancer and Hamartoma syndromes.

Although the mechanisms by which nutrient and stress cues regulate mTORC1 are not yet clear, as diagrammed in [Figure 3](#) we now know a great deal regarding the regulation of mTORC1 by circulating growth factors and hormones. At the heart of these signalling pathways lie the tuberous sclerosis proteins, TSC1 (hamartin) and TSC2 (tuberin). These proteins function together in a complex and act as a GAP (GTPase-activating protein) for the small GTPase Rheb. Although the details are still debated, GTP-loaded Rheb is thought to bind and activate mTORC1. TSC2 is phosphorylated by several kinases and these phosphorylation events either activate or inhibit its GAP activity. In this manner, TSC2 integrates signal

inputs from several upstream regulators to generate an environmentally appropriate level of mTORC1 activity.

Although potentially many membrane receptors act upstream of TORC1, signalling pathways emanating from the [▶insulin receptor](#) are illustrated here ([Fig. 3](#)). Insulin receptor ligation results in the binding and phosphorylation of insulin-receptor substrate (IRS). Phosphorylated IRS subsequently recruits additional signalling molecules which ultimately results in the activation of [▶phosphatidylinositol 3 kinase \(PI3K\)](#) and the [▶small GTPase Ras](#).

Activated Ras triggers signalling down the Raf-MEK1/2-Erk1/2-RSK1 [▶MAP kinase cascade](#). This process is inhibited by the RasGAP neurofibromin (NF1). Both Erk1/2 and RSK1 can directly phosphorylate TSC2 and these phosphorylation events inhibit the GAP activity of TSC2 and thus ultimately promote mTORC1 activity.

Activated PI3K converts phosphatidylinositol-4,5-phosphate (PIP2) to phosphatidylinositol-3,4,5-phosphate (PIP3). Accumulation of this lipid recruits two kinases, PDK1 and PKB/akt to the plasma membrane. This process is antagonized by the lipid phosphatase PTEN. At the plasma membrane, PKB is activated via phosphorylation by both PDK1 and mTORC2. PKB has many roles in the cell and there are several mechanisms by which PKB-derived signals can inhibit TSC2. (i) Like Erk1/2 and RSK1, PKB can directly phosphorylate and inhibit TSC2. (ii) PKB also promotes nutrient import and thus is required to maintain cellular energy levels. High cellular energy levels (i.e., a high ATP:AMP ratio) blocks activation of the [▶AMP-dependent kinase, AMPK](#). AMPK is itself activated by an additional kinase, LKB1, and when active, AMPK phosphorylates TSC2 at a residue that stimulates its GAP activity. (iii) PKB can also phosphorylate and inhibit glycogen synthase kinase 3 (GSK3). GSK3 is a component of the [▶Wnt signalling](#) pathway. When active, GSK3 can phosphorylate TSC2 that has been 'primed' by previous phosphorylation by AMPK. Phosphorylation by GSK3 further activates TSC2 GAP activity.

As summarized in [Table 1](#), an important theme has emerged from these recent studies: many negative regulators of mTORC1 are known tumor suppressors while many positive regulators of mTORC1 are known proto-oncoproteins. Importantly, genes encoding many of these negative regulators are found mutated in patients suffering from [▶hamartoma syndromes](#). These patients suffer from benign tumors that on occasion progress to malignant disease. These observations suggest that mTORC1 may be a good target to treat hamartoma syndromes and possibly certain [▶cancers](#).

Pharmacological Intervention

Although novel mTOR inhibitors are in development, to date, only the mTORC1-specific inhibitor rapamycin (and closely related derivatives that improve

TOR Signalling. Table 1 Protooncogenes and tumor suppressors in the mTOR signalling network

Protooncogenes	Evidence
PI3K	Elevated PI3K has been observed in a variety of human cancers and is implicated in transformation and tumor progression
PKB/akt	Akt is amplified in a subset of human cancers
Ras	Mutations yielding hyperactive Ras protein are frequent in human cancers
Rheb	Elevated in many tumor cells, Rheb may be the critical target by which farnesyl transferase inhibitors inhibit tumor growth
S6K1	S6K1 is overexpressed in many breast cancers and this is correlated with poor prognosis
Wnt	Wnts are secreted signalling molecules best characterized for their roles in development. The Wnt signalling pathway is also implicated in cancer
Tumor suppressors	Name and description of corresponding hamartoma syndrome
NF1	neurofibromatosis type 1; benign and malignant peripheral nerve sheath tumors
PTEN	Cowden disease, Bannayan–Riley–Ruvalcaba syndrome, Proteus syndrome, Lhermitte–Duclos disease; overgrowths of various organs, hamartomas of the skin, mucosa, cerebellar cortex; high risk of developing several types of cancer. Spontaneous pten mutations found frequently in human cancers
LKB1	Peutz–Jeghers syndrome; hamartomas in the gastrointestinal tract
TSC1,2	Tuberous sclerosis complex; hamartomas of the brain, heart, kidneys, skin, lungs, and eyes

pharmacokinetic properties) are used clinically or are being evaluated in clinical trials. Rapamycin analogs (►[rapalogs](#)) under evaluation include: CCI-779, Wyeth; RAD001, Novartis; and AP-23573, Ariad. These drugs are not without side effects. Oral mucositis often limited the dose that could be administered in phase I trials. Other side effects include metabolic malfunction leading to deviations from normal serum cholesterol and triglyceride levels, anemia and diarrhea. At present, it appears that mTORC1 inhibitors work most effectively when used in combination with other compounds as discussed below. Although not discussed here, it is clear from [Figure 3](#) that there are many additional drug nodes, particularly upstream of mTORC1, which could be targeted for potential clinical benefit. Also, as noted below, compounds that directly or indirectly activate mTORC1 may in some circumstances have clinical value.

Clinical Uses of Rapamycin and Rapalogs

Allograft Rejection and Autoimmune Disorders

Rapamycin has been known for many years to possess immunosuppressive activity by interfering with the activation of B- and T-cells by ►[interleukin-2](#). Indeed the first clinically approved indication for rapamycin was renal transplantation. Currently, rapamycin and RAD001 also show promise in liver transplantation and cardiac transplantation, respectively. Generally, treatment protocols utilize a combination of an mTORC1 inhibitor, a ►[calcineurin](#) inhibitor and steroids to optimize immunosuppression and minimize nephrotoxicity and other side effects. Rapalogs are also

being evaluated as agents to treat autoimmune diseases including rheumatoid arthritis, psoriasis, multiple sclerosis, and Parkinson's disease.

Cancer and Hamartoma Syndromes

The recent links made between mTORC1 and tumor suppressors/oncoproteins suggests that aberrantly high mTORC1 activity may be the underlying cause of hamartomas and perhaps some cancers. Based on this, many clinical trials are underway to determine the efficacy of rapalogs as anti-cancer agents. Current phase III clinical trials include:

CCI-779 – renal carcinoma, lymphoma

AP23573 – sarcomas

RAD-001 – pancreatic cancer

Again, several preclinical studies suggest that rapalogs will have synergistic effects when used in combination with other chemotherapeutics.

Cardiovascular Disease

At present, the most widespread clinical use of rapamycin is in the prevention of in-stent restenosis. Following balloon angioplasty, stents (an expandable metal coil) are often inserted into the coronary artery. Stenting improves outcome, but in a significant percentage of patients, smooth muscle cells may eventually invade and proliferate within the stent resulting in a reblockage of the artery (in-stent restenosis). Since their approval in 2003, rapamycin-eluting stents are now commonly used to stop the growth/proliferation of invading smooth muscle cells and thus prevent this form of in-stent restenosis.

Muscle Hypertrophy/Atrophy

mTORC1 signalling plays a critical role in the regulation of postnatal muscle size. Both muscle hypertrophy and muscle atrophy can have pathophysiological consequences. For example, cardiac hypertrophy is a major risk factor for heart failure. Interestingly, due to an inherited defect in APMK, persons suffering from Wolff–Parkinson–White syndrome present with hypertrophic cardiomyopathy. Presumably, this defect results in hyperactivation of mTORC1 in cardiac tissue. Conversely, many diseases, for example cancer and HIV/AIDS, or simply a sedentary lifestyle, can lead to muscle atrophy. In extreme cases, such as cancer ►cachexia, patients are physically weakened to a state of immobility. Thus rapalogs may have potential in treating muscle hypertrophic disorders while mTORC1 agonists (or a TSC2 GAP inhibitor) could have clinical value in treating muscle atrophic disorders.

Metabolic Disorders

Recently, a ‘feedback loop’ in mTORC1 signalling has been described. As shown in Figure 3, the mTORC1 substrate S6K1, in addition to regulating translation, also phosphorylates and thus antagonizes the function of IRS. Inhibition of IRS function reduces insulin response. A reduction in insulin response, also known as insulin resistance is typically associated with both type 2 ►diabetes and obesity. Thus, although not yet tested, rapalogs may in the future be useful reagents to treat metabolic disorders involving insulin resistance.

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QT syndrome and as a side effect of the action potential prolonging drugs.

►Antiarrhythmic Drugs

Total Sleep Time

Synonyms

TST

Definition

The sum of all periods of sleep throughout the night. Used as a measure of diagnosing and recovery following treatment for *insomnia*.

►Sleep

Toxicogenomics

Toxicogenomics is a form of analysis by which the activity of a particular toxin or chemical substance on living tissue can be identified based upon a profiling of its known effects on genetic material. Toxicogenomics may also be of use as a preventative measure to predict adverse “side”, i.e. toxic effects, of pharmaceutical drugs on susceptible individuals. This involves using genomic techniques such as gene expression level profiling and single-nucleotide polymorphism analysis of the genetic variation of individuals.

►Gene Expression Analysis and Microarray Technology

Trace Amines

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Synonyms

Biogenic amines

Torsade de Pointes

Torsade de pointes is a life-threatening polymorphic ventricular tachycardia, which occurs in inherited long

Definition

Trace amines are a family of endogenous monoamine compounds including β -phenylethylamine (PEA), p-tyramine (TYR), tryptamine (TRP) and octopamine (OCT). The trace amines share close structural similarity with the well known classical monoamine neurotransmitters such as dopamine (DA), norepinephrine (NE) and serotonin (5-HT). As their name suggests, trace amines occur in comparably much lower abundance than monoamine neurotransmitters. For historical reasons, other endogenous amine compounds which might share some structural similarities with PEA, TYR, TRP or OCT are not referred to as trace amines.

Basic Characteristics

The synthesis and metabolism of trace amines and monoamine neurotransmitters largely overlap [1]. The trace amines PEA, TYR and TRP are synthesized in neurons by decarboxylation of precursor amino acids through the enzyme aromatic amino acid decarboxylase (AADC). OCT is derived from TYR by involvement of the enzyme dopamine β -hydroxylase (Fig. 1; DBH). The catabolism of trace amines occurs in both glia and neurons and is predominantly mediated by monoamine oxidases (MAO-A and -B). While TYR, TRP and OCT show approximately equal affinities toward MAO-A and MAO-B, PEA serves as preferred substrate for MAO-B. The metabolites phenylacetic acid (PEA), hydroxyphenylacetic acid (TYR), hydroxymandelic acid (OCT), and indole-3-acetic (TRP) are believed to be pharmacologically inactive.

The rate of synthesis is similar for trace amines and monoamine neurotransmitters, however, trace amines undergo a more rapid turnover due to their higher affinity to MAO and the lack of comparable cellular storage. Thus, the tissue concentration of trace amines in the vertebrate central nervous system is estimated to be in the range of 1–100 nM, depending on the trace amine and brain area, in contrast to micromolar concentrations of classic monoamine neurotransmitters.

Due to their physicochemical properties trace amines can pass the cell membrane to a limited extent by passive diffusion, with the more lipophilic PEA and TRP crossing membranes more readily than the more polar amines TYR and OCT. In spite of these features, trace amines show a heterogeneous tissue distribution in the vertebrate brain, and for TYR and OCT storage in synaptic vesicles as well as activity-dependent release have been demonstrated. So far, trace amines have always been found co-localized with monoamine neurotransmitters, and there is no evidence for neurons or synapses exclusively containing trace amines.

Trace Amine-Associated Receptors

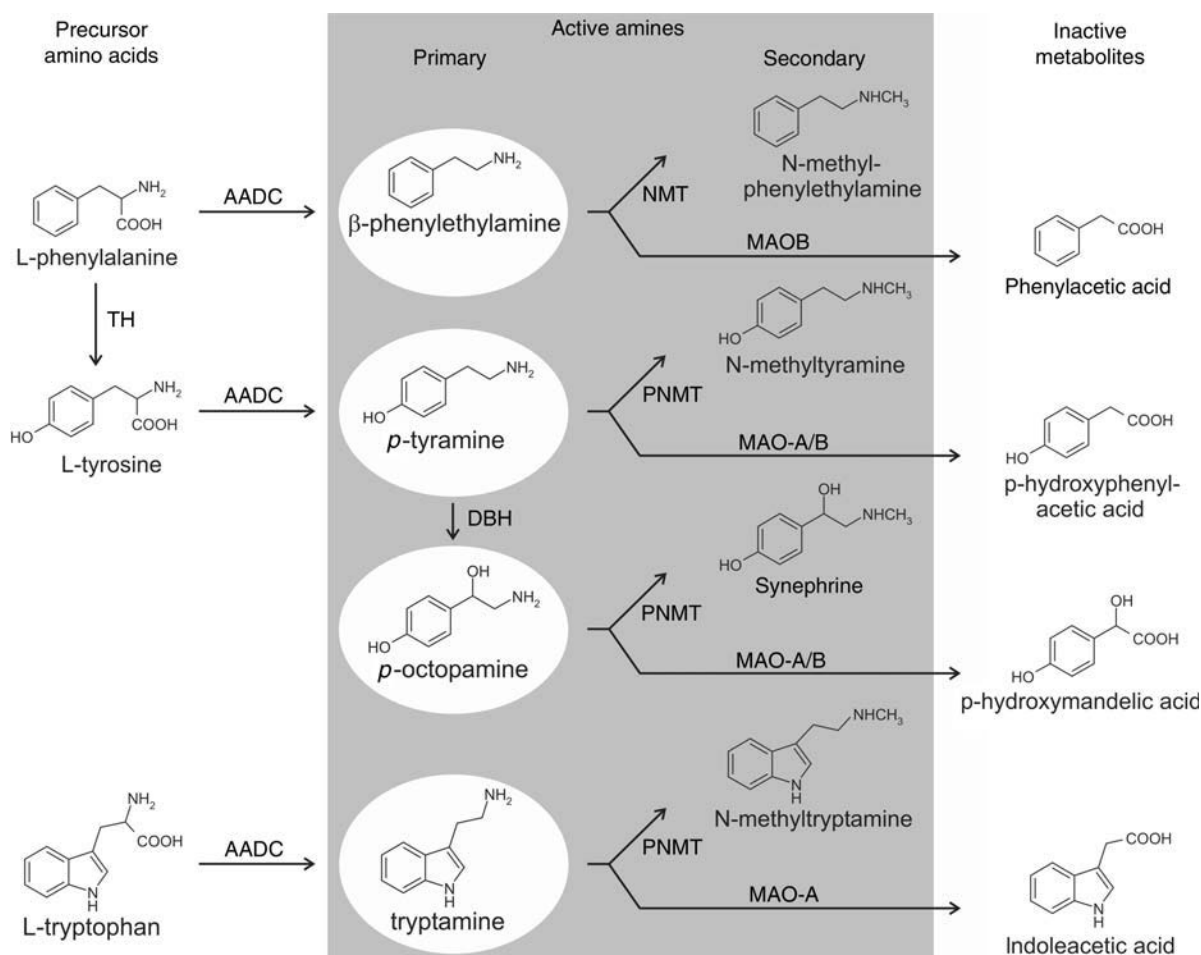
Although trace amine-selective receptors had been hypothesized for several decades and trace amine binding sites had been reported earlier, it was only

in 2001 that the first members of the Trace Amine-Associated Receptor (TAAR) family were identified [2]. While only two members of the TAAR family were confirmed trace amine-sensitive and synthetic ligands for two additional receptors were recently reported (Table 1) [4], the majority of TAARs are currently orphan receptors. As recent data suggest that not all TAARs are sensitive to trace amines, and as some TAARs respond to a range of compounds different from trace amines, the receptor family has been officially designated as Trace Amine-Associated Receptors [3].

Phylogenetically, the TAARs most likely evolved from a common single ancestor gene through sequential **gene duplication** and speciation (Gene specification refers to the mutational events underlying the divergence of orthologous genes, i.e., two gene counterparts present in two different species which are derived from a single ancestral gene.) events, which occurred independently from other GPCR families (Fig. 2). By comparison to other monoamine-sensitive receptors such as the 5-HT sensitive GPCRs, individual *TAAR* genes show a high degree of sequence identity between species. However, the TAAR family reveals pronounced species differences with respect to the total number of *TAAR* genes and the proportion of **pseudogenes** in each species [3]. While there are 19 and 16 *TAAR* genes including 2 and 1 pseudogenes in rat and mouse, respectively, only 9 TAAR genes are represented in the human and chimpanzee genomes, out of which 3 (human) and 6 (chimpanzee) are pseudogenes. It is believed that the difference with respect to the total number of genes and the degree of pseudogenization reflects the species-specific physiological roles of the receptors. All *TAAR* genes map to a single, narrow chromosomal region of ~100–200 kb, and the orientation of the *TAAR* genes on the chromosomes is conserved between human, chimpanzee and rodents. This pattern as well as **pharmacophore modeling** studies, suggests the existence of three receptor subfamilies of so far unknown functional relevance.

Trace amine-sensitive receptors also occur in invertebrates such as the fruitfly (*Drosophila melanogaster*) or the nematode *Caenorhabditis elegans* and in both cases are activated by TYR and OCT. However, these invertebrate trace amine receptors evolved independently, are phylogenetically not related to the TAAR family, and show more similarity to the vertebrate 5-HT, DA and **adrenergic receptors**. In zebrafish (*Danio rerio*) 57 *TAAR* genes and 40 pseudogenes have been reported. The evolution of *TAAR* genes in zebrafish through tetraploidization, block duplication and local duplication events is more complex than for most other GPCR families studied so far.

With respect to tissue distribution, the available data is limited to mRNA transcripts which, for all TAARs studied so far, are present at generally low levels when compared to e.g., 5-HT and DA receptors. The most



Trace Amines. Figure 1 The main routes of trace amine metabolism. The trace amines β-phenylethylamine (PEA), *p*-tyramine (TYR), octopamine (OCT) and tryptamine (TRP), highlighted by white shading, are each generated from their respective precursor amino acids by decarboxylation. They are rapidly metabolized by monoamine oxidase (MAO) to the pharmacologically inactive carboxylic acids. To a limited extent trace amines are also *N*-methylated to the corresponding secondary amines which are believed to be pharmacologically active. **Abbreviations:** AADC, aromatic amino acid decarboxylase; DBH, dopamine β-hydroxylase; NMT, nonspecific *N*-methyltransferase; PNMT, phenylethanolamine *N*-methyltransferase; TH, tyrosine hydroxylase.

detailed information is available for TAAR1 which for human was detected in several brain areas involved in mood regulation and memory formation such as the amygdala, hypothalamus, hippocampus and cerebellum, as well as in several peripheral tissues including liver, pancreas, lung, kidney and skeletal muscle [2]. Recently, the expression of TAAR2–9 in the mouse ►olfactory epithelium was described. The presence of at least three TAARs sensitive to volatile amines (Table 1) present in urine suggests a role of TAARs for social interaction in rodents [4].

Function of Trace Amines

Prior to the identification of specific receptors, the understanding of trace amine functions on the molecular level was limited to hypotheses mainly considering the following possibilities [1]: (i) trace amines may serve

as co-transmitters to monoamine neurotransmitters such as DA, NE or 5-HT and, linked to this assumption, (ii) trace amines may function as so-called false neurotransmitters by interfering with the well known receptor systems and transporters of monoamine neurotransmitters; alternatively, (iii) trace amines may function as neurotransmitters or neuromodulators independently of catecholamines through an independent receptor system which at that time remained to be identified. Currently, three modes of action for trace amines can be distinguished: (i) amphetamine-like activities linked to the monoamine neurotransmitter system (e.g., interaction with DA transporter), (ii) TAAR-dependent function (s) of trace amines as neuromodulators, and (iii) the TAAR-dependent role of trace amine-like compounds in the context of olfaction and social communication in rodents.

Trace Amines. Table 1 The trace amine-associated receptor (TAAR) family

Subgroup	Unified nomenclature	Previous name(s)	Selected ligands
1	TAAR1	TA1 ^{a,b} , TRAR1 ^{a,b,c} , TAR1 ^{a,b,c}	PEA, TYR, TRP, OCT, synephrine, amphetamine, 2-methylbutylamine
	TAAR2	GPR58 ^{a,c}	–
	TAAR3	GPR57P ^a , GPR57 ^c	Cyclohexylamine, 2-methylbutylamine, isoamylamine
	TAAR4	TA2P ^a , 5-HT4P ^a , TA2 ^b	PEA, TYR
2	TAAR5	PNR ^a	Trimethylamine, <i>N</i> -methylpiperidine
3	TAAR6	TA4 ^{a,b} , TRAR4 ^a , TAR4 ^a	–
	TAAR7	–	–
	TAAR7a	TA8 ^b	–
	TAAR7b	TA12 ^b	–
	TAAR7c	–	–
	TAAR7d	TA15 ^b	–
	TAAR7e	TA14 ^b	–
	TAAR7f	TA13P ^b	–
	TAAR7g	TA9 ^b	–
	TAAR7h	TA6 ^b	–
	TAAR7i	–	–
	TAAR8	TA5 ^{a,b} , TRAR5 ^a , TAR5 ^a GPR102 ^a	–
	TAAR8a	TA11 ^b	–
	TAAR8b	TA7 ^b	–
	TAAR8c	TA10 ^b	–
	TAAR9	TA3 ^{a,b} , TRAR3 ^a , TAR3 ^a	–

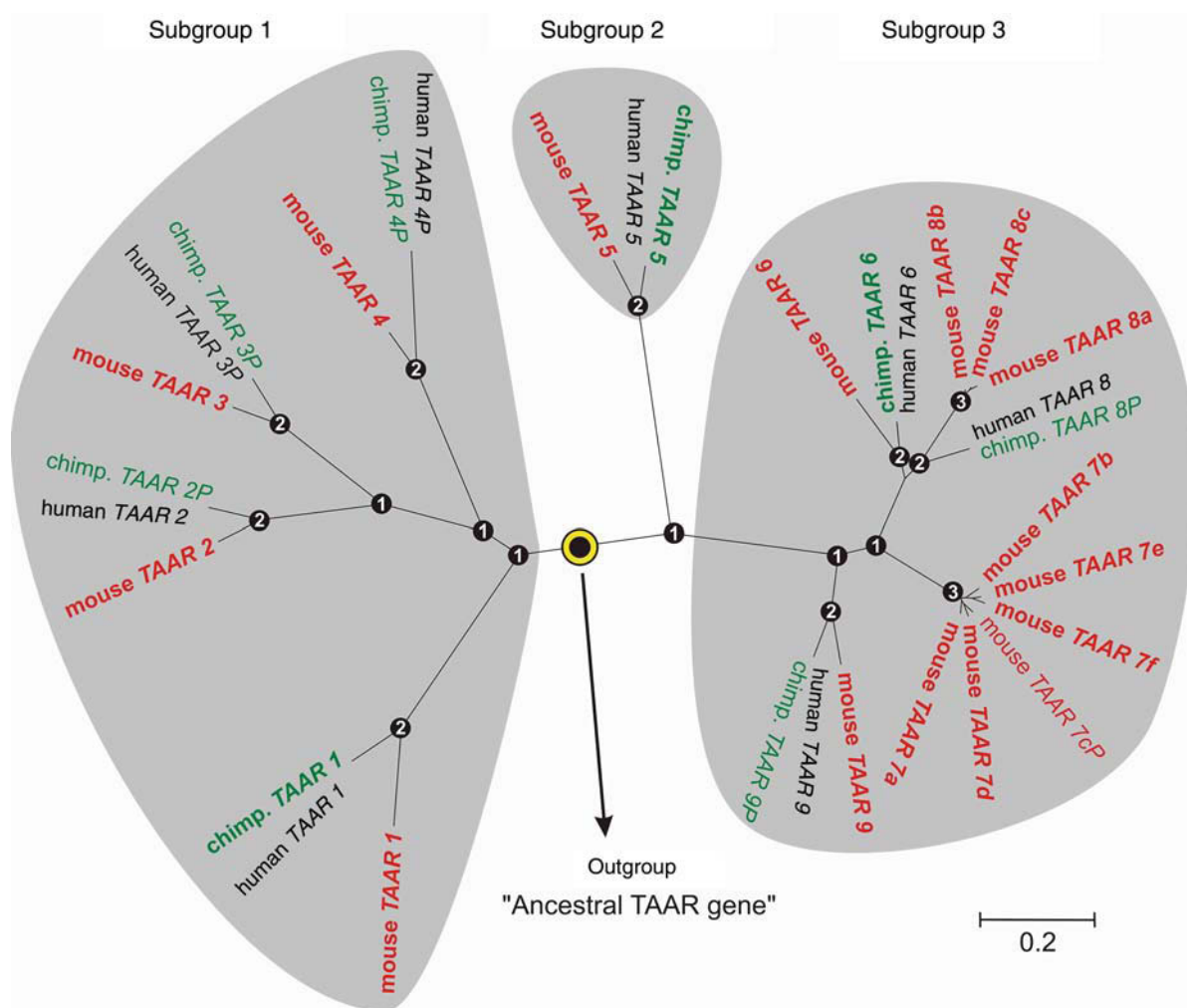
^aNomenclature previously used in human.^bNomenclature previously used in rat.^cNomenclature previously used in mouse.

The amphetamine-like properties of trace amines are best described for PEA which shares close structural similarity to amphetamine and can displace monoamine neurotransmitters from ►[synaptic vesicles](#) and trigger their release into the synaptic cleft by acting on the dopamine transporter. However, this effect is only observed at high, supra-physiological PEA concentrations and thus might not occur under physiological conditions.

The role as neuromodulators is supported by observations that trace amines at physiological concentrations on their own do not alter the electrical excitability of neurons but instead modulate the neuronal response to catecholamine neurotransmitters. Further insight into this mode of action comes from a recent study involving a TAAR1-deficient mouse mutant [5]. These mice display a grossly normal behavioral profile in the absence of pharmacological or behavioral challenges, but the psychostimulant and TAAR1 agonist amphetamine triggers a much stronger increase in the locomotor activity and extracellular DA levels in certain brain areas in TAAR1-deficient as compared to wild-type mice. This

phenotype relates to neurochemical observations from these mutant mice indicating that TAAR1 negatively modulates the release of dopamine from neurons and possibly also the signaling of dopamine receptors. So far, it is not known which molecular mechanisms link TAAR1 to dopamine release and signaling, and whether TAAR1 or maybe also other TAARs are linked only to dopamine or also to other neurotransmitters (Fig. 3).

The relevance of the TAAR receptor system for olfaction and social communication in rodents is supported by the reported expression of several TAARs in olfactory sensory neurons and their sensitivity towards volatile amine compounds which are present in urine [4]. Notably, sensitivity of the TAARs expressed in the olfactory epithelium towards the traditional four trace amines has not been observed, except for TAAR4 and expression data suggest that the trace amine-sensitive TAAR1 is not expressed in the olfactory epithelium. The role of TAARs in the context of olfaction in rodents is only beginning to emerge and so far, there are no data on the exact physiological impact of these mechanisms in the living animal.



Trace Amines. Figure 2 Phylogenetic relationship of TAARs. The phylogenetic relationship of human, chimpanzee and mouse TAAR genes. The TAAR gene family most probably evolved from a common ancestor gene sharing closest similarity to the human gene encoding the 5-HT₄ receptor by a series of gene duplication and speciation events: (1) gene duplication before the rodent and human lineage split; (2) speciation leading to separate primate and rodent lineages; and (3) gene duplication within the rodent lineage. The phylogenetic relationship, the conserved orientation of TAAR genes on the chromosomes, as well as modeling data suggest three separate TAAR subgroups with currently unknown functional relevance. The chimpanzee and human genes basically do not separate in the phylogenetic scheme as they share sequence identities in the range of >97–99%. Pseudogenes are indicated by the suffix “P.”

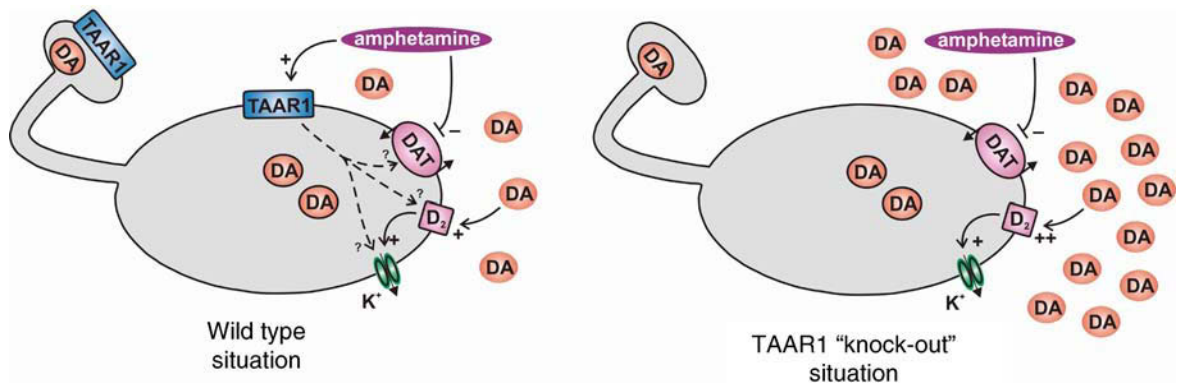
The TAAR receptor system has also been associated with body temperature regulation on the basis of putative thyroid hormone metabolites and their synthetic derivatives (thyronamines) activating TAAR1 in rodents. However, as these effects are only observed with thyronamine concentrations several orders of magnitude above physiological levels, and as the specificity of these compounds has not been determined, the physiological significance of these observations is unclear.

Invertebrates contain the trace amines OCT and TYR, which are the invertebrate counterparts of the vertebrate adrenergic transmitters NE and adrenaline. Invertebrate OCT and TYR occur at much higher

concentrations, act through trace amine-sensitive receptors different from the TAARs, and have different functions than in vertebrates.

On the molecular level, all TAARs for which ligands are available, couple to Gas, at least in recombinant systems. Links to other signaling pathways as well as potential heterodimerization within the TAAR family or with other GPCRs have so far not been observed. All TAAR genes have a very similar size of about 1 kb, and [posttranslational modification](#) and subcellular trafficking of the receptors are both not well understood.

It should be noted that current knowledge on the TAAR-mediated function of trace amines is mainly derived



Trace Amines. Figure 3 Modulation of dopaminergic neurotransmission through TAAR1. An amphetamine challenge applied to mice triggers the release of dopamine (DA) which is reflected in a transiently increased locomotor activity. This behavioral effect is mediated by the increased activation of DA receptors on neurons, notably of the D_2 receptors which is the overall most abundant of the DA receptors in the brain. In TAAR1 deficient "knock-out" mice the same amphetamine challenge triggers a much stronger DA release corresponding to a much stronger increase in locomotor activity as compared to the wild-type animals. These observations have prompted the conclusion that TAAR1 provides a generally negative modulation to DA signaling in the rodent central nervous system. The exact mode of interaction between TAAR1, the D_2 and potentially other DA receptors, the DA transporter or any downstream signaling element is currently not known. Abbreviations: DA: dopamine; D_2 : D_2 dopamine receptor; DAT: dopamine transporter; K^+ : potassium channel; TAAR1: trace amine-associated receptor 1.

from studies on TAAR1 in rodents and recombinant systems. Basic information such as the tissue distribution in human, rodents or other species is not yet available for the majority of TAARs, and there is no data on a possible function of TAARs outside the nervous system. Thus, the current understanding of the TAAR receptor family and the TAAR-dependent functions of trace amines is incomplete and can not be extrapolated to the TAAR/trace amine system as a whole.

Trace Amines and Diseases

Based on several observations linking trace amines to the modulation of catecholamine neurotransmitter systems it is not surprising that trace amines have been implicated in most neuropsychiatric disorders that are associated with these systems. Alterations in trace amine function are thought to be involved in the etiology of a variety of neuropathological conditions, including major depression, stress/anxiety disorders, schizophrenia, bipolar disorder, anorexia/eating disorders, attention deficit hyperactivity disorder (ADHD), drug abuse/dependence, migraine/cluster headache, epilepsy, and Parkinson's disease. As the link to trace amines for some indications is predominantly indirect, the discussion in this context is focused on depression and schizophrenia.

Trace amines, most notably PEA, have been implicated in the etiology of depression. It has been suggested that trace amines stabilize mental functions within physiological limits, a hypothesis which might be in line with the assumed role of trace amines as

neuromodulators on the cellular level. Earlier studies examining the levels of PEA in a variety of body fluids have in general supported this so-called PEA hypothesis, which postulates that a deficit in the level or turnover of PEA is underlying the etiology of depression, while an excess might result in manic episodes. The PEA hypothesis receives support from clinical observations that administration of PEA or its precursor L-phenylalanine produces a relief from depression symptoms in depressed patients. Furthermore, a reduction of PEA levels in cerebrospinal fluids of depressed patients as compared to healthy subjects has been reported to correlate with the disease state. It is believed that the increase in trace amine levels triggered by the application of MAO inhibitors is an important molecular mechanism underlying the antidepressant effect of these inhibitors. Moreover, MAO-B deficient mouse mutants, which show increased PEA levels in the central nervous system, display a behavioral profile reminiscent of animals treated with traditional antidepressants. Taken together, these observations support a significant role of trace amines in the regulation of affect and mood.

The postulation of a possible role of trace amines in the context of schizophrenia was kindled early on by the structural similarity between PEA and amphetamine. Symptoms such as hallucinations and paranoid episodes caused by a prolonged amphetamine intoxication are reminiscent of patients suffering from acute schizophrenia. Further support for a role of trace amines in the context of schizophrenia comes from clinical studies

reporting increased PEA plasma levels in patients suffering from acute schizophrenia, as well as elevated urinary excretion of PEA in paranoid schizophrenics. Moreover, several studies identified a chromosomal region comprising the TAAR genes as schizophrenia susceptibility locus. These findings from human subjects are in agreement with the observation that TAAR1-deficient mice reveal a deficit in a rodent behavioral model for schizophrenia symptoms known as ►[prepulse inhibition of the startle response](#) [5].

The knowledge of the potential link between trace amines and pathological conditions is only beginning to emerge. The understanding of the physiological roles of trace amines and TAARs in vivo will much depend on the arrival of powerful pharmacological tools such as highly specific TAAR agonists and antagonists.

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TRAF

TNF Receptor Associated Factors.

- [TNF Receptor Superfamily](#)
- [Table appendix: Receptor Proteins](#)

Trafficking

Trafficking is controlled movement of a protein from one subcellular location to another.

Tranquilizers

Tranquilizers (also called antianxiety drugs) are used to treat a variety of psychiatric disorders which go along with anxiety (anxiety disorders). Serotonin-reuptake inhibitors and the benzodiazepines are the most commonly employed drugs for the treatment of common clinical anxiety disorders.

- [Benzodiazepines](#)

Transactivation

Activation of transcription by the binding of a transcription factor to a DNA-regulatory sequence.

- [Nuclear Receptors](#)
- [Transcriptional Regulation](#)

Transcription Factor

Transcription is the process by which information encoded in DNA is “transcribed” into RNA, another form of genetic information. The enzyme RNA polymerase (RNA Pol) catalyzes the synthesis of RNA from the DNA template. In prokaryotes there is only one RNA pol, while there are three distinct RNA pol enzymes in eukaryotes. A general or basal transcription factor (TF) is a protein that mediates the binding of RNA pol and the initiation of a basal level of transcription. The complex consisting of RNA POL and the associated general transcription factors required for a basal level of transcription is called the basal transcription machinery. A regulatory transcription factor typically binds DNA regions distal (5') to the site of initiation of transcription. These factors modulate basal levels of transcription (positively or negatively) by a mechanism thought to involve DNA bending or looping such that the regulatory TFs bound at sites distal to the site of initiation interact with the basal transcription machinery to affect transcriptional activity of the gene.

- [Nuclear Receptors](#)
- [Transcriptional Regulation](#)

Transcriptional Coactivators

Transcriptional co-activators form a bridge between transcription factors, such as NF- κ B and AP-1 and general transcription factors (GTFs), and RNA polymerase II. They do not bind DNA themselves but enable the transduction of the regulatory signal (e.g. NF- κ B) to the basal transcriptional complex, enabling initiation of gene transcription. There are two types of coactivators, ubiquitous or general coactivators and more specialised coactivators that are tissue-specifically expressed and/or specifically bind to certain transcription factors. Coactivators contain histone acetyltransferase activity.

Coactivators enhancing the transcriptional activity of steroid hormone receptors include SRC-1 (steroid-receptor co-activator 1) or TIF2 (transcriptional intermediary factor 2), which are recruited by the DNA/steroid hormone receptor complex. Their main role is to attract other transcriptional coactivators with histone acetyltransferase activity in order to decondense chromatin and allow for the binding of components of the general transcription apparatus.

- Contraceptives
- Glucocorticoids
- Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
- Transcriptional Regulation

Transcriptional Regulation

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Synonyms

Regulation of mRNA synthesis; Regulation of gene expression

Definition

Transcriptional regulation encompasses the modulation of the synthesis rate of mRNA due to cell type specific factors that may be triggered by external stimuli.

Basic Mechanisms

Background

Cellular phenotypes and their responses to environmental, hormonal, and growth-promoting stimuli depend

on changes of protein function. The functional properties of proteins can be modulated by chemical modifications. Equally important is the composition of the protein mix of a cell due to alteration in their abundance. Abundance is determined by the balance of new synthesis and turnover. Both synthesis and turnover are regulated and changed under the influence of extracellular stimuli. The rate of protein synthesis can be influenced on several levels: the gene encoding the protein is controlled during the process of transcriptional initiation and elongation. The mRNA thereby synthesized is subjected to turnover, which again can be differentially regulated. Most pre-mRNAs carry intron sequences that need to be excised from RNA before translation. The excision of some introns is not obligatory but is regulated (alternative ► [splicing](#)) and can generate different RNA-species from a single gene. Finally the rate of translation can be modulated. All these options coexist in cells of the organism. It is impossible to say at this time which type of regulation of gene expression predominates as most recent discoveries have revealed new regulatory steps, e.g. by microRNAs, and additional features are likely to be revealed in the future. We restrict the discussion here to transcriptional initiation because its complexity is best studied and most extracellular stimuli affect transcriptional initiation at the promoters of genes. Promoters assemble numerous regulatory proteins, which integrate the input of different stimuli.

The history of transcriptional regulation reaches back to 1961 when the first regulatory proteins were proposed on the basis of genetic experiments with bacteria. Nutrients stimulate bacterial gene expression. It took 20 additional years to discover regulatory components in mammalian cells, which select genes and regulate transcription in response to hormonal and other extracellular stimuli. The mechanistic role of chromatin in transcriptional regulation has been recognized much more recently and still is a matter of intensive research. Most recent and ongoing work aims at unravelling the enormous network of protein–protein interactions and their dynamic nature, which determine gene expression. To present a taste of the complexity of transcriptional regulation we choose here interleukin-6 (*IL-6*) gene activity, which is increased upon inflammatory stimuli and downregulated by anti-inflammatory drugs.

Transcriptional Initiation

A specialized multi-subunit enzyme, ► [RNA polymerase II](#), catalyzes transcription of mammalian coding genes such as that for IL-6. The resulting messenger RNA is complementary to one strand of the double-strand DNA molecule. To be able to assemble the nucleotide building blocks according to the base pairing rules, the double strand must be locally unwound to expose the coding strand. This is one of the functions

exerted by RNA polymerase II. Upon proper alignment of the nucleotides in the form of highly energized triphosphates, they are coupled to each other in the process of phosphodiester formation. The RNA polymerase moves in a step-wise fashion releasing the single-stranded RNA as RNA-polymerization grows in the 5' to 3' direction. Several RNA polymerase molecules can travel along the gene at the same time, one after the other.

Although we will stick to the *IL-6* ►gene, it should be mentioned at the side that two other RNA polymerases exist in mammalian cells responsible for the synthesis of ►RNA molecules, which are not translated into proteins: ribosomal (rRNA), transfer (tRNA), small nuclear (snRNA), small nucleolar (snoRNA), and some of the recently discovered microRNAs and piRNAs. These RNA molecules act in the process of translation and mRNA turnover. Micro and piRNAs are probably extremely important in the definition of stem cells and of differentiation programs. Some of them are synthesized by RNA polymerase II.

Protein-encoding genes are dispersed in an excess of noncoding DNA. An important question is therefore, how does RNA-Polymerase II find a gene and, once found, how does it know where to start. The start site of transcription was discovered by comparing sequences upstream of many coding genes. In defined distance 5' of the first coding nucleotide, most genes carry a sequence resembling TATA where RNA polymerase II binds. So-called ►general transcription factors guide the RNA polymerase to this site, e.g. the TATA binding protein TBP, which is associated with numerous cofactors, so-called TBP associated factors (TAFs). One of the TAFs with a molecular weight of 250 kDa (TAFII250) appear to be a jack-of-all-trades in that it organizes many steps: assembly of other TAFs, catalyzing the formation of the general transcription factor of polymerase II D (TFIID), which comprises the TBP, interaction with acetylated lysine residues in core histones (see later), and exerting enzyme activities (protein kinase, ubiquitin-activating/conjugating, and acetylase activities). The factors involved in transcriptional initiation bind in a sequential order: bound TFIID allows the recruitment of the basal transcription factors TFIIA and TFIIB followed by the binding of RNA polymerase II. All this can take place if the DNA sites are accessible, a problem to be discussed subsequently.

Given that these proteins have properly assembled, the initiation complex is ready to start transcription. How does the enzyme get started? A component of TFIID, again a multi-subunit complex: TFIIH, unwinds the DNA and phosphorylates serine-5 of the C-terminal tail (CTD) of the largest polymerase subunit (Rpb1). Serine-5 phosphorylation and phosphorylation of serine-2 (by pTEFb) are required to release the enzyme from the other components of the initiation complex and to start RNA synthesis.

Regulation of Transcriptional Initiation

If recruitment of polymerase II would just occur in the presence of general transcription factors, transcription should start from all TATA containing promoters at a constant rate which is, however, not the case at all. Of the thousands of genes, the *IL-6* gene is selected for transcription only under conditions of inflammation. These conditions obviously determine at which promoter to start transcription. In addition, transcriptional initiation is not just a TATA finding exercise. Rather, the genes are heavily packed in ►chromatin, which forms an obstacle to proteins searching a DNA sequence. Both, the specific selection of the gene to be transcribed and the “unpacking” (chromatin remodeling), is achieved by proteins called transcription factors or enhancer factors, which recognize so-called “enhancer” sequences. These are specific for the gene, but can be in respectable distance to the TATA sequence. Enhancer-binding proteins recruit the chromatin remodeling enzymes that open the chromatin. One may ask how do enhancer factors themselves get access to the DNA. The basal structures forming the chromatin, the nucleosomes, assembled by histones, are not localized randomly on DNA but are deposited in a manner directed by specific sequence elements. It is believed that unfolding occurs in several steps one of which exposes nucleosome-free sections. The entry of one enhancer factor channels the assembly of others, further modifying the chromatin histones and thereby unfolding the chromatin.

The assembly of the enhancer factor is the essential step at which transcriptional regulation occurs. The factors binding to the enhancer sequences are not necessarily active at all times, but may require an activating modification. Once bound, they can serve to assemble so-called coactivators, necessary to enhance transcription, or they may recruit corepressors, blocking transcription. Enhancer sequences are often assembled in larger conglomerates, which can thus bind several enhancer factors. Several transcriptional activators and repressors can therefore act on one and the same gene. Their integrated activities define the synthesis rate of the mRNA.

Enhancer-Binding Factors in Regulatory Units of the *IL-6* Promoter

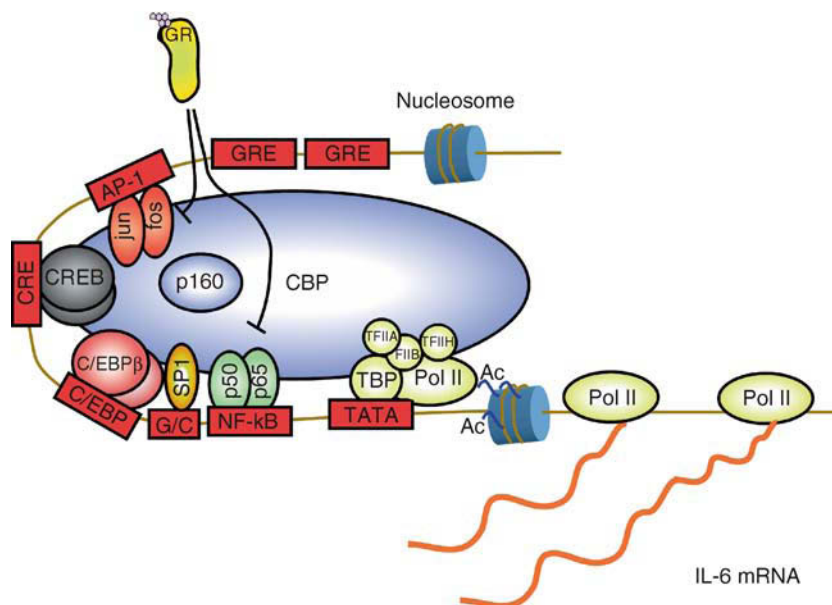
The regulatory aspects eluted to above, can be illustrated best by looking at expression of a specific gene. We chose as an example the gene encoding the cytokine *IL-6*. *IL-6* is a peptide that has a wide range of biological activities and regulates a number of important physiological and pathological processes, e.g. the activity of the immune system, but also oncogenesis. The gene can be expressed in many tissues of the body. Whereas under most conditions virtually no *IL-6* mRNA or protein can be detected, *IL-6* production is rapidly induced if cells come

into contact with specific stimuli such as lipopolysaccharides (constituents of the outer cell wall of bacteria), other cytokines (IL-1, TNF, IL-2, IFN β), or growth factors (PDGF). These factors stimulate the activation and binding of sequence-specific enhancer factors, which find their proper (cognate) sequences in the promoter of the *IL-6* gene.

A sequence stretch 300 base pairs upstream of the transcriptional start site suffices for most of the transcriptional regulation of the *IL-6* gene (Fig. 1). Within this sequence stretch several transcription factors find their specific recognition sites. In 5' to 3' direction, AP-1, CREB, C/EBP β /NF-IL6, SP-1 and NF- κ B can bind to the promoter followed by TATA and its TATA binding protein TBP. Most enhancer factors become active in response to one or several different stimuli and the active factors can trigger transcription individually or in concert. For example, AP-1 is active upon cellular stress, or upon stimuli that tell cells to proliferate; CREB becomes also active if cells experience growth signals, but also upon elevation of intracellular levels of cyclic adenosine monophosphate (cAMP), which occurs upon stimulation if so called hormone-activated G protein-coupled receptors.

How do these transcription factors recognize their binding sites? They contact DNA through the major groove where recognition of a specific DNA sequence is provided by interactions of amino acid side chains of α -helical domains or β -sheets of the factor with individual base pairs. The tertiary structures of enhancer factors provide the optimal contact to the major groove. A small number of characteristic domain motifs serve to mediate the specific recognition of DNA sequence: helix-turn-helix motifs, homeodomains, helix-loop-helix domains, and Zn-fingers each consisting of α -helices and/or β -strands that are stabilized by the Zn-ion, which coordinates Cys- and His-residues. The interaction between enhancer factor and DNA can force the DNA into a curved and twisted conformation.

It is believed that many transcription factors bind DNA as dimers ([▶dimeric transcription factors](#)) in either the same or opposite orientation. The DNA sites therefore can look like two direct repeat sequences or like palindromes. The interaction of the subunits with each other must obviously be specific and be mediated by [▶dimerization](#) domains. Specificity and stability of the dimers (dimeric transcription factors) is mostly promoted by hydrophobic or ionic interphases, e.g. a



Transcriptional Regulation. Figure 1 Schematic representation of the occupancy of the promoter of the *IL-6* gene by sequence-specific transcription factors, coactivators, general transcription factors, and RNA-Polymerase II. Enhancer sequences are shown in boxes such as GRE (glucocorticoid responsive element), AP-1 (AP-1 binding site binding the Jun:Fos AP-1 dimer), CRE (CREB responsive element binding the CREB dimer), C/EBP (binding C/EBP dimers), G/C (GC rich boxes binding SP1), and the NF- κ B (binding dimeric p50:p65 complexes). Sequence-specific transcription factors interact with coactivators (p160, CBP) to facilitate acetylation on the histone tails (Ac) and to interact with general transcription factors (TBP, TFIIA, TFIIB, TFIIF), which are sequentially recruited to allow effective binding of RNA polymerase II (Pol II) to the promoter and start with mRNA synthesis. The glucocorticoid receptor (GR) can interfere with the transcription stimulating activity of sequence-specific transcription factors such as AP-1 or NF- κ B.

basic leucine zipper motif oriented to one side of an amphipathic helix, as for the transcription factor complex AP-1. While in this case the hydrophobic amino acid residues stabilize the interaction, polar amino acids of the α -helices determine the interaction specificity. AP-1 turned out to represent a whole family of heterodimeric factors composed of subunits from three protein families, Jun, Fos, and ATF [1]. The polar amino acid code enforces that Fos proteins (c-Fos, FosB, Fra-1, Fra-2) can only heterodimerize with Jun proteins (c-Jun, JunB, JunD) while Jun proteins can also **dimerize** with members of the ATF family: e.g. Fos:Jun; Jun:ATF2. The dimer composition dictates recognition of DNA sequence elements specific for the **dimer**. For instance, Fos:Jun recognizes a DNA sequence element different from that selected by Jun:ATF2. Different dimers (**dimeric transcription factors**) of the same family can exert opposite functions: e.g. Fos:Jun heterodimers activate genes, while cJun:JunB heterodimers usually suppress the same gene.

As mentioned above, many transcription factors are not always active. Rather the activity of transcription factors is often achieved by induced reversible modification. Most frequently is the addition of phosphate groups (phosphorylation) to Ser, Thr, or Tyr residues. For the AP-1 component c-Jun the phosphorylation at Ser63 and Ser73 enhances activity when cells are subjected to stress, e.g. radiation. Phosphorylation is, however, dispensable for c-Jun-dependent tissue homeostasis in the liver, indicating that certain activities do not require the regulatory enhancement. Jun-N-terminal kinase and a kinase called RSK or p38 catalyze the phosphorylation of AP-1.

The cAMP responsive element binding factor (CREB) is also activated by phosphorylation. Depending on the stimuli, CREB is the target of a cAMP dependent protein kinase or of kinases called MAPKs, RSK, and CamKIV. As in AP-1, CREB carries a basic leucine zipper motif (bZIP), which mediates homo **dimerization** of CREB when bound to the CRE.

Similarly, the CCAAT/enhancer-binding protein beta (C/EBP β) can also form homo- and heterodimers with different other factors.

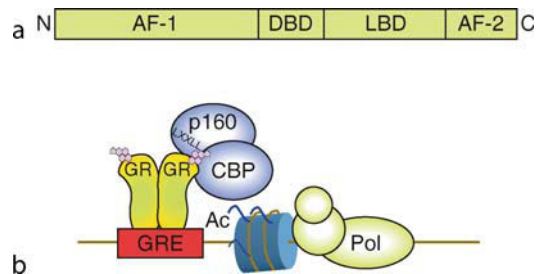
SP-1 family members bind to a GC rich sequence between the C/EBP and NF- κ B binding sites. SP-1 is important for basal expression of house keeping genes as well as for inducible gene expression.

Essential for induction of the *IL-6* gene in inflammatory reactions is the binding site for nuclear factor kappa B (NF- κ B). NF- κ B responds to cytokines, stress, free radicals, ultraviolet irradiation, and bacterial, viral, or even parasitic antigens [2]. NF- κ B stands for a family of subunits, which form homo-, and heterodimers. All NF- κ B proteins share a highly conserved DNA-binding/dimerization domain called the Rel homology domain (RHD) consisting of two β -strand core domains

connected by a flexible link. NF- κ B is retained in the cytoplasm as long as an internal inhibitor (I- κ B) masks the nuclear uptake signal. The inhibitor is removed by proteasomal degradation, which is triggered by phosphorylation through an I- κ B kinase complex. The extracellular stimuli determine which kinase and which NF- κ B subunits are activated. For instance, the so-called canonical pathway activates NF- κ B consisting of RelA and p50.

The fact that a promoter comprises a given transcription factor recognition sequence, does not prove that this sequence element is of any importance. The IL-6 promoter carries such a putatively irrelevant element. In the most 5' region of the IL-6 promoter sequence two consensus sequences of glucocorticoid responsive elements (GREs) have been described. GREs in principle can bind the glucocorticoid receptor (GR), a transcription factor which, like NF- κ B, is retained before hormonal stimulation in the cytoplasm in association with chaperones and transported into the nucleus upon binding of the ligand, cortisol. The GR belongs to the nuclear receptor super family whose 48 members share a common arrangement of their ligand-binding (LBD), Zinc-finger-motif-containing DNA-binding (DBD), and transactivation domains (Fig. 2) [3].

The strict control of the activity of nuclear receptors by their respective ligands has recently permitted to follow the dynamics of DNA occupancy and to discover a surprising feature, which may be true also for other enhancer-binding factors. During transcriptional activation these transcription factors do not stably reside



Transcriptional Regulation. Figure 2 The action of the glucocorticoid receptor dimer on positive regulated promoters. (a) Schematic representation of the domain structure from the N to C terminus of the glucocorticoid receptor containing activator function 1 (AF-1), DNA-binding domain (DBD), ligand-binding domain (LBD), and activator function 2 (AF-2). (b) The glucocorticoid receptor (GR) binds as a homodimer to the palindromic sequence (glucocorticoid responsive element, GRE) and interacts with chromatin remodeling complexes, which are comprised by CREB-binding protein (CBP) and members of the p160 histone acetylase family. The p160 histone acetylases interact via their LxxLL motifs with the ligand-activated AF-2 domain of the GR.

on their target site, but rather associate and dissociate in a cyclic manner. The hypothesis is that this dynamic behavior is essential for the efficient initiation of transcription.

Instead of activating transcription the cortisol-induced GR represses IL-6 synthesis and, even more surprisingly, repression does not involve the GRE elements, but rather the κ B site (Fig. 1). It appears that of a monomeric GR protein without itself touching the DNA interacts with the RelA component of NF- κ B [3]. As a result GR blocks the action of NF- κ B. The negative interference by this crosstalk is not restricted to NF- κ B, it occurs also with AP-1 and CREB, and with several other transcription factors not relevant for IL-6 expression. A nuclear isoform of the LIM protein Trip6 mediates the interaction between these factors and is required for the inhibitory GR function. This interesting negative crosstalk is part of the immune-suppressive action of cortisol.

Coactivators/Corepressors

We have discussed previously the factors, which select promoters and their enhancer sequences. The critical question, how can the binding of transcription factors to promoters influence transcription, remains to be considered. Most insight came from the analysis of ligand-induced transcription factors such as the GR. The transcription factors assemble large complexes of several proteins, coactivators [4]. They are thought to stabilize the complex transcription machinery or to modify and loosen the local chromatin. Two general classes of chromatin-remodeling factors have been identified, which appear to play critical roles in transcriptional activation: ATP-dependent nucleosome remodeling complexes (exemplified by SWI/SNF), and factors that exert histone acetyltransferase (HAT, e.g. p160) activity. They are addressed by different transactivation domains of the GR, for instance: SWI/SNF by the N-terminal so-called AF-1, p160 by the C-terminal AF-2 domain. AF-2 undergoes enormous conformational changes following ligand binding to the GR and is not accessible without ligand interaction. p160 stands for a family of related **coactivators** (SRC-1, SRC-2/GRIP1/TIF2, RAC3/ACTR/p/CIP), which share a nuclear-receptor interaction motif (LXXLL) (Fig. 2). These coactivators in turn are capable of recruiting additional chromatin-modifying enzymes such as CBP/p300 and the histone arginine methylase CARM1. The two related molecules CBP (first identified as CREB binding protein) and p300 (first identified as interacting protein of the transforming adenoviral oncoprotein E1A) have intrinsic functions involved in transcriptional coactivation: (i) they create a physical bridge from various DNA-bound transcription factors (AP-1, NF- κ B, GR, CREB, C/EBP, p53 and others) and coactivators (p160 family members and others) to the basal transcriptional machinery

(TBP-RNA polymerase); (ii) they are potent **histone acetyl transferases** inducing chromatin remodeling (loosening).

Beside coactivators so-called corepressors exist that are bound to transcription factors such as nuclear receptors and inhibit the initiation of transcription. These factors include the nuclear receptor corepressor (NCoR) and the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT), which interact with nuclear receptors and serve as platforms for complexes containing **histone deacetylases (HDACs)**. These enzymes cause the reversal of histone acetylation of histones leading to a tightening of chromatin and enhancing its inaccessibility for RNA polymerase containing complexes.

Chromatin Modification

The core unit of the chromatin, the nucleosome, consists of histones arranged as an octamer consisting of a (H3/H4)₂-tetramer complexed with two histone H2A/H2B dimers. Accessibility to DNA-binding proteins (for replication, repair, or transcription) is achieved by posttranslational modifications of the amino-termini of the histones, the histone tails: phosphorylation, acetylation, methylation, ubiquitination, and sumoylation. Especially acetylation of histone tails has been linked to transcriptional activation, leading to weakened interaction of the core complexes with DNA and subsequently to decondensation of chromatin. In contrast, deacetylation leads to transcriptional repression. As mentioned above, transcriptional coactivators either possess HAT activity or recruit HATs. HDACs in turn act as corepressors.

The transfer of acetyl groups to lysine residues in the histone tails occurs in a reversible manner allowing quick acetylation and deacetylation and thus rapid changes in transcriptional regulation [5]. **Methylation** of lysine or arginine residues by **methyl-transferases** and the recently demonstrated **demethylation** by histone demethylases involve a higher degree of complexity, since single lysine residues can be mono-methylated, di-methylated, or tri-methylated and arginine residues can carry two methyl groups in a symmetric or asymmetric manner. One modification appears to determine subsequent other modifications. Combinations of the possible histone modifications create a histone code with distinct consequences for transcription. For instance, methylation of histone H3 tail lysine-4 (K4) promotes acetylation of K9, K14, K17, and K23 by CBP. K9 methylation in contrast inhibits acetylation of these lysines probably leading to recruitment of the potent transcriptional repressor heterochromatin protein-1 (HP1).

Other steps of RNA synthesis such as elongation or termination also control the abundance of proteins. These are less well understood than the initiation of transcription described here.

Pharmacological Relevance

As transcription is central to cellular phenotypes, its manipulation could be of therapeutic value for a number of diseases including chronic inflammation and cancer. The use of ligands, which directly interact with a transcription factor and activate or inactivate the factor, is an elegant case of transcriptional interference. Ligands for nuclear receptors have been the first widely applied agents targeting transcription: e.g. estrogens, retinoic acids, and glucocorticoids. More recent pharmaceutical attempts to inhibit the interactions between transcription factors with their coactivators by small molecules have yet been successful in only a few cases. For example, interference with the interaction between the transcription factor p53 and the coactivator CBP hinders the transcriptional activation of p53-dependent target genes, which trigger cell death. As an interesting other approach the abundance of a transcription factor can be modulated by specifically blocking degradation, again exemplified by small molecules inhibiting the binding of Mdm2 to p53, the first step which targets p53 for degradation. Elevated p53 can be desirable if cancer cells are to be destined for cell death.

The modulation of transcription nicely demonstrates how results of basic science can be translated into medical application. These few examples of drugs in successful use stimulate at the same time the intensive study of gaps in our understanding of transcriptional control, hoping to find new specific ways of interference.

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Transcriptome

The entire population of mRNA transcripts in the cell, weighted by their expression levels.

► [Gene Expression Analysis](#)

Transcriptome Analysis

► [Gene Expression Analysis](#)

Transduction

Transduction is the introduction and expression of genes in a cell.

Transfection

Transfection is the introduction of DNA or RNA into eukaryotic cells in vitro and in vivo.

► [DNA Vaccination and Genetic Vaccination](#)

Transforming Growth Factor-Beta

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Synonyms

Transforming growth factor-beta receptor signaling pathway; TGF-beta receptor signaling pathway; TGF-β receptor signaling pathway

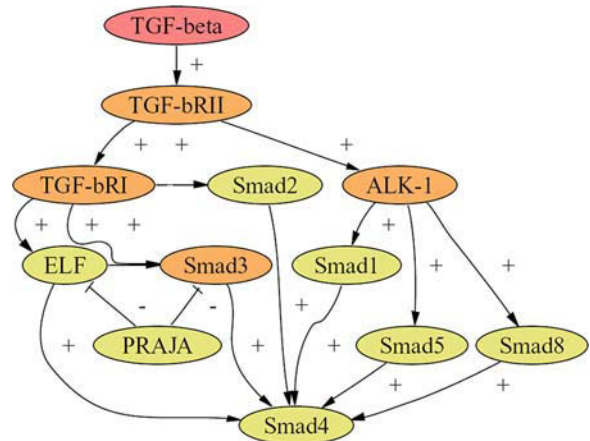
Definition

Transforming growth factor-beta (TGF-β) proteins are multifunctional ► [morphogens](#) that control cell proliferation, differentiation and apoptosis, as well as cell migration and immune surveillance. TGF-β acts as a tumor suppressor, but can also act as a tumor promoter in

later metastatic cancers. Additionally, TGF- β signaling plays an important role in wound healing and fibrosis. TGF- β signals are transmitted through type I and II serine/threonine kinase receptors to specific intracellular mediators, known as ►Smad proteins. Activation of Smads by receptor-mediated phosphorylation results in nuclear translocation and activation of gene expression. Adaptor proteins such as ►SARA, ►filamin, Disabled, CrkL, and ►embryonic liver fodrin (ELF), are important components of the TGF- β signaling pathway. Additionally, E3 ►ubiquitin ligases including ►SMURFs, SCF/Roc1, ►ectoderm, ►itch, and ►PRAJA are key regulators of this pathway. In the gastrointestinal system, disruptions and errors in TGF- β signaling lead to tissue-specific cancers such as hepatocellular cancers, gastric adenocarcinomas, and colonic adenocarcinomas. Thus, adaptor protein interactions and ubiquitin-dependent protein degradation in the TGF- β /Smad pathway present important new molecular targets for these difficult-to-treat cancers. Recent research has led to the development of a number of small molecule compounds that target the TGF- β pathway and may offer new therapies for these and other cancers [1–4].

Basic Characteristics

TGF- β signals are conveyed through Type I (T β RI) and Type II (T β RII) serine/threonine kinase receptors to specific intracellular mediators, the Smad proteins. There are at least eight different Smad proteins in vertebrates that fall into three classes: (i) Receptor-activated Smads (R-Smads): Smad 1, Smad 2, Smad 3, Smad 5, and Smad 8; (ii) Co-mediator Smad: Smad 4; and (iii) Inhibitory Smads (I-Smads): Smad 6 and Smad 7. Activation of the TGF- β receptor complex by ligand binding results in the phosphorylation of T β RI by the serine/threonine kinase activity of T β RII. R-Smads, such as Smad 2 and Smad 3, act as direct substrates of specific activated T β RI, and are phosphorylated on the last two serines near the carboxyl terminus. Once phosphorylated, R-Smads associate with the co-mediator Smad, Smad 4, and then translocate into the nucleus. In the nucleus, Smad complexes activate specific genes such as *integrins* and *E-cadherin* through cooperative interactions with other DNA-binding and co-activator proteins. The R-Smads and Smad 4 (predominantly cytoplasmic) are modulated by adaptors such as SARA and ELF. Adaptor proteins play critical roles in controlling Smad access to receptors, to components of other signaling pathways, and possibly for translocation into the nucleus. Subsequently, TGF- β functions, such as growth, differentiation, vascular remodeling, and cell fate specification are facilitated by adaptor protein interactions. Studies are now emerging that demonstrate prominent role of the TGF- β pathway in gastrointestinal cell/progenitor cell



Transforming Growth Factor-Beta. Figure 1

Transforming growth factor- β signaling pathway. The transforming growth factor- β (TGF- β) signaling pathway shown occurs through varying mechanisms, yet the receptor-II/receptor-I-mediated pathway has been the most extensively studied. Signals in this pathway are transmitted through serine/threonine kinase receptors located at the cell surface to the Smad proteins, which are specific intracellular mediators. The activation of these Smad proteins results in their nuclear translocation and consequent gene expression activation. From Kitisin et al, Science STKE (Connections Map, ►[http://stke.sciencemag.org/cgi/cm/stkecm; CMP 17699](http://stke.sciencemag.org/cgi/cm/stkecm;CMP_17699)).

formation as well as its complex role in differentiation, with disruptions resulting in cancer (Fig. 1).

Adaptor and Receptor Interacting Proteins

Smad proteins reside in the cytoplasm, and upon stimulation, translocate to the nucleus as part of an oligomeric complex. Smad 2/3 and Smad 4 are thought to be distributed along the microtubule (MT) network, and MT stability may be involved in Smad inactivation. Thus, microtubules represent a large group of TGF- β signaling adaptor proteins. Additional adaptor proteins include SARA, ELF, Filamin, CrkL, FKBP12, Disabled-2 (Dab2), Drosophila inhibitor of apoptosis (DIAP)-1 and -2, T β RI-associated protein (TRAP)-1 and -2, T β RII binding WD-domain protein (TRIP-1), X-linked inhibitor of apoptosis (XIAP), Daxx, AMSH-2, and CD2-associated protein (CD2AP).

SARA is a scaffolding protein that regulates the subcellular localization of inactivated R-Smads, potentially scaffolding the TGF- β receptor kinase to the Smad 2 substrate. Filamins are a family of actin polymerization proteins that also form scaffolds for a range of signaling proteins including SAP kinases such as MKK-4, small GTPases Rho and Ras, as well as Smad 2 and Smad 5.

ELF, a β -Spectrin, is a key component of TGF- β signaling that functions to recruit Smads to the receptor by controlling the subcellular localization of Smad 3 and Smad 4. Interestingly, ELF does not appear to interact with SARA or filamin, and in *elf* mutants, SARA and filamin distribution is the same as in wild-type mice. Thus, TGF- β signaling through R-Smad/ELF interactions may work by way of a different mechanism than that of SARA and filamin.

Extrinsic regulatory pathways such as the Erk, JNK, and p38 MAP kinase pathways also contribute to Smad regulation. In addition, there is a Ca^{2+} -dependent interaction between calmodulin (CaM) and several Smad family members. CaM binds to the N-terminal half of Smad 2 with subsequent phosphorylation inhibiting TGF- β induced nuclear import and transcriptional activity of Smad 2. Therefore, CaM influences Smad protein function in response to agents that regulate intracellular Ca^{2+} flux. Other TGF- β induced signaling pathways involve activation of Rho-like GTPases, including RhoA, Rac and cdc42 through RhoA-specific guanine exchange factor or Ras activation. Thus, elucidation of ELF phosphorylation by TGF- β receptors as well as cross-talk with the stress activated protein kinases is of great interest.

TGF- β Signaling and Wound-Healing

TGF- β has a significant role in wound healing [5]. As platelets arrive at a wound location, they release cytokines, which recruit inflammatory cells, neutrophils, and macrophages. Through a positive feedback mechanism, these cells proceed to secrete additional cytokines, including TGF- β . In Smad 3 mutant mice, TGF- β signaling is disrupted and there is a reduced recruitment of inflammatory cells at the site of epithelial injury. Macrophages digest the extracellular matrix as part of the wound healing process. TGF- β suppresses the proteases released by macrophages, thus allowing for greater progression of the proliferative phase of wound repair. Angiogenesis is another important aspect of the proliferative phase and is essential to normal wound healing. Angiogenesis is promoted by subcutaneous injection of TGF- β . Fibroblasts also secrete TGF- β using a positive feedback mechanism. TGF- β causes the proliferation of fibroblasts which function in wound healing by producing extracellular matrix. Additionally, TGF- β is a strong chemoattractant. In animal models, topical application of TGF- β to wounds produces mixed results because of the presence of proteases and variability of receptor expression. In some instances, TGF- β has been shown to substantially increase the production of both collagen and fibrinogen. TGF- β signaling through Smad 3 is pivotal for collagen synthesis, myofibroblast activation, wound contraction, and scar formation. Interestingly, Smad 3 null mice

have accelerated wound repair without scar formation. TGF- β is responsible for this conversion, and it does not occur if TGF- β signaling is disrupted. As the role of TGF- β signaling in wound healing continues to unravel, many therapeutic targets for improving wound healing are anticipated.

TGF- β Signaling and Malignancy

Despite the large number of molecules found to interact with the TGF- β pathway, few have been identified as functional tumor suppressors using either mouse or human genetics. The TGF-beta pathway is inactivated in nearly all gastrointestinal cancers, from TBR11, TBR1, Smad 2, and Smad 4. Recent exciting data have revealed the role of ELF, a key TGF- β pathway adaptor, in hepatocellular cancer suppression.

Smad 4 is located within human chromosome 18q21.1 and is deleted or mutated in 40–50% of pancreatic cancers and 30% of colorectal cancers. Heterozygous germline mutations in Smad 4 are also responsible for a subset of familial juvenile polyposis (FJP), a disorder characterized by predisposition to hamartomatous polyps and gastrointestinal cancer. Evidence for the role of haploinsufficiency of Smad 4 was demonstrated by the discovery that tumors in FJP individuals almost always retained the wild-type allele of Smad 4. Although continued expression of Smad 4 was not demonstrated, this prompted analysis of the effects of *Smad 4* haploinsufficiency in a mouse model. *Smad 4*^{+/-} mice were found to develop hyperplasia of the fundus and antrum and interestingly, intercrosses with *elf* mutants exacerbated the phenotype, further highlighting, the role of ELF haploinsufficiency in suppression against gastric cancers. Interestingly, *▶elf*^{+/-}/*Smad 4*^{+/-} mutants also develop colonic adenomas. Hence, ELF appears to have a tumor suppressor action on its own in hepatocellular cancer, whereas with Smad 4 presents a synergistic role which is conceivably more akin to the natural development of sporadic gastric cancers in humans, than the total loss of both alleles of one gene.

Growth inhibition by TGF- β , associated with inhibition of c-myc, cdks, reduction in cyclin D1 levels, and inhibition of cdk-4-associated Rb kinase activity, as well as induction of cdk inhibitors p15 and p27, has been noted in intestinal epithelial cells. Loss of responsiveness to growth inhibition from TGF- β occurs in many cell types including: breast, colorectal carcinoma, and pancreatic carcinoma cells. Mutational inactivation of TBR11 represents one mechanism of this process, which in many cases, leads to the development of gastrointestinal cancer. Thirteen percent of colorectal carcinomas are thought to be associated with a replication error (RER) or microsatellite instability phenotype. Subsequent inactivation of TBR11 and

restoration of T β RII results in decreased tumorigenicity. Accordingly, mutations with loss of function of Smad 2 and Smad 4 have been noted in 4% and 30% of human colorectal cancers, respectively. APC Δ^{716} mice intercrossed with mice heterozygous for Smad 4 develop larger and more invasive colorectal tumors. Furthermore, Smad 4 is required for gut endoderm lineage, and *Smad 4*^{+/-} mice develop gastric tumors after 12 months. Also, Smad 2 is needed for gastrulation, and Smad 3 is needed to establish the gastrointestinal mucosal immune response to TGF- β signals. Hence, Smad 3-deficient mice frequently develop gut abscesses and die between 1 and 10 months because of impaired mucosal immunity. Defective liver development with loss of gastrointestinal epithelial cell shape and polarity is also observed in *elf*^{-/-} homozygotes, and *Smad 2*^{+/-}/*Smad 3*^{+/-} double heterozygous mice.

Loss of ELF in human gastric cancer is a significant aspect of gastric tumor suppression by TGF- β . Two lines of evidence indicate that ELF is biologically and functionally important in suppressing gastrointestinal tumors. First, hepatic cancers are only observed in mice with *elf* mutations, and have not been observed in other TGF- β signaling pathway or interacting molecules. Secondly, Smad 3 homozygote knockouts do not develop cancers, and Smad 4 homozygote mutants are early embryonic lethals observed in mutants gastric cells that express normal levels of Smad 3 and Smad 4 do not exhibit E-cadherin localization and substrate independent cell-cell adhesion. Importantly, the RING-H2 domain-containing E3 ubiquitin ligase, PRAJA, is a key regulator of ELF expression, and could represent an oncogenic pathway in gastric and hepatocellular cancer by virtue of PRAJA ubiquitination of ELF and Smad 3.

Drugs

We have gained considerable insight into the therapeutic potential of this protein through the use of TGF- β antagonists and transgenic mice with defective TGF- β signaling and we have evaluated the potential toxicity of TGF- β modulation and its overall efficacy in treating cancer. FC Soluble Type II Receptor

Antagonist (SR2F), a chimeric protein of the Fc IgG and extracellular domain of T β RII, and 1D11, a TGF- β neutralizing antibody, have not demonstrated toxicity. Importantly, SR2F, the T β RII antagonist has revealed strong antimetastatic activity.

The tumor suppressive role of TGF- β has been well documented, and disrupting TGF- β signaling can potentially promote tumorigenesis. On the other hand, TGF- β can also promote tumorigenesis, especially during the later growth and metastatic phases. Because of this, there must be caution taken as to identifying when the tumorigenic role of TGF- β signaling is prevalent. To that end, mouse models of cancer have been developed that allow the tissue-specific conditional knockout of T β RII. Through this animal model, the role of TGF- β in tumor progression can be analyzed, and the development of TGF- β antagonists aimed at treating specific stages of tumor development can be observed.

TGF- β overexpression is especially significant in breast cancer as TGF- β -mediated growth inhibition becomes inactive in breast cancer cells. However, in a mouse model of metastatic breast cancer, it was demonstrated that TGF- β has tumor suppressive characteristics in the primary lesion while promoting metastatic dissemination. Because of this observation, it can be concluded that TGF- β signaling can be altered to influence metastasis and effective therapeutics for inhibiting cancer growth can be generated. Examples are one such the development of TGF- β signaling inhibitors such as antisense oligonucleotides and monoclonal antibodies such as those listed in Table 1.

Current agents have focused on inhibiting TGF- β signaling. Lerdelimumab and Metelimumab are monoclonal antibodies that bind to TGF- β , and are being evaluated for their ability to reduce post-operative scarring and suppress fibrosis, respectively. A novel approach utilizing a vaccine to TGF- β_2 is being tested as a therapeutic for non-small-cell lung cancer. Likewise, AP 12009, a TGF- β_2 antisense oligonucleotide, is being evaluated for its ability to suppress a variety of metastatic tumors. Small molecule inhibitors of the TGF- β pathway, such as LY550410 and

Transforming Growth Factor-Beta. Table 1 Agents that target the TGF- β signaling pathway

Agent name	Type	Target	Indications
Lerdelimumab	Monoclonal antibody	TGF- β_2 and TGF- β_3	Postoperative scarring in glaucoma patients
Metelimumab	Monoclonal antibody	TGF- β_1	Renal fibrosis
Belagenpumatumucel-L	Anti-TGF- β_2 vaccine	TGF- β_2	Nonsmall-cell lung cancer
AP 12009	Antisense oligonucleotide	TGF- β_2	Glioma, pancreatic carcinoma, melanoma
LY550410	Small molecule	T β RI kinase domain	Preclinical
SB-505124	Small molecule	T β RI kinase domain	Preclinical

SB-505124, both of which target the T β RI kinase domain, are in preclinical use. An alternative approach to inhibiting TGF- β signaling is to focus on the tumor suppressive function of this pathway. Promising future treatments will focus on pathways that are activated when the TGF- β signaling tumor suppressor pathway is inactivated. Examples are inhibitors to wnt signaling, CDK4 activation and IL-6.

► Neurotrophic Factors

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Transforming Oncogenes

The growth of tumours can be regarded as the result of an accumulation of genetic changes in a wide variety of genes, the incorrect activation of oncogenes and the loss of function of tumour suppressor genes. Oncogenes are defined on the basis of their gain of function. The number of oncogenes isolated since about the end of the 1970s has grown to over 250.

► Antisense Oligonucleotides

Transgene

A transgene is an additional extra gene which is introduced into the germline of an animal.

► Transgenic Animal Models

Transgenic Animal Models

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Definition

Transgenic animals are genetically engineered animals, which allow the functional assessment of specific genes or proteins in health and disease.

Description

It is a major goal in biomedical research to identify control elements that regulate complex physiological functions, such as regulation of blood pressure or behavior. These molecular control elements are of interest since they represent potential therapeutic targets for various disease states. Three different strategies have been employed to assess gene functions in terms of providing the determinants for a particular phenotype: (i) Analysis of naturally occurring mutations in humans and animals, (ii) random chemical mutagenesis with subsequent screening for phenotypes and (iii) the generation of transgenic and targeted mutant animals, which are the subject of this contribution. In these approaches, an additional gene (“►transgene”) is transferred to an animal or an inherent gene is mutated in order to assess the respective phenotypes. For the purpose of clarity, a clear distinction between “transgenic” (i.e., nontargeted) and “targeted mutant” animals is made here, although many scientists use the term “transgenic” in a broader sense to encompass all genetically engineered animals.

Generation of Transgenic Animals

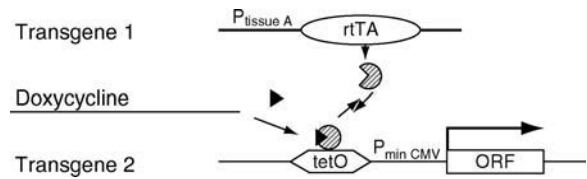
Transgenic animals express a foreign gene, the “transgene,” which is typically introduced into the mouse germline by microinjection of DNA into fertilized eggs. This technique is applicable to mice, rats and other species. The DNA will be integrated at random, frequently as concatamers. The tissue distribution and the level of expression vary between mouse lines depending on integration sites and copy number, even when the same DNA was used for injection. Transgenic mice may constitutively and tissue-specifically overexpress the transgene, or may carry gain-of-function mutations in the transgene. Alternatively, they may also contain dominant negative mutations, ribozyme, RNAi or antisense constructs. Thus, both gain-of-function and loss-of-function approaches can be pursued with transgenes.

Generation of Targeted Mutant Animals

For the ►targeted mutant approach, targeting vectors are constructed that contain DNA sequences derived from the gene to be targeted. They are specifically integrated at the desired genomic location by homologous recombination in murine embryonic stem cells. These cells are then injected into ►blastocysts, which in turn are reimplanted into pseudopregnant foster mothers. The embryonic stem cells contribute to the developing embryo, which may carry the mutation in its germ line. This technique is currently available only for mice. The target gene may be disrupted by introduction of a neomycin resistance cassette or by deletion of one or more, or even all, exons (classical or global ►knockouts). Since regulatory elements in the neomycin resistance cassette may affect the expression of neighboring genes, it is advisable to eliminate this cassette e.g., by cre/loxP-mediated recombination. Knockout mutations may also be studied in the heterozygous state to assess potential gene dosage effects [1]. The phenotypic consequences of the knockout mutation are expected to provide information on the normal function of the respective gene in wild type animals. Targeted mutagenesis is not limited to gene knockouts. Virtually any desired subtle mutation, e.g., a point mutation, or replacement of a gene with another gene, can be introduced into the mouse germline in this way (“knock-in”). The potential advantage of introducing a point mutation using a “knock-in” approach, compared to a transgenic approach, is that with a gene-targeted point mutation, the gene harboring the mutation is expressed under the control of the endogenous promoter. In contrast, in transgenic approaches the expression level and expression pattern may be different, making it more difficult to interpret a potential phenotype. In general, targeted point mutations permit more precise modeling of many human disease mutations.

Tissue-Specific and Inducible Transgenes

The expression of transgenes in time and space is dependent on the promoter used in the transgene construct and the site of DNA integration. Many transgenes are expressed constitutively, which may perturb development and even cause lethality. To avoid these problems, transgenes may be expressed in an inducible fashion. Mice carrying an inducible transgene grow up normally. The acute effect of transgene expression can then be reversibly induced in adult animals. The same animal may be studied before and after the expression of the transgene and thus serve as its own control, e.g., in behavioral studies. Several systems have been developed to achieve inducible expression, of which the tet system appears to be the most widely used (Fig. 1). The reverse tetracycline-controlled transactivator (rtTA) consists of a rtTA fusion protein



Transgenic Animal Models. Figure 1

Tissue-specific inducible transgene expression using the rtTA system. To achieve an inducible tissue-specific expression of a transgene, a mouse carrying two transgenes may be generated: Transgene 1 codes for rtTA, driven by a promoter expressed in tissue A ($P_{\text{tissue A}}$). Transgene 2 contains the open reading frame (ORF) of the gene that is to be expressed inducibly, preceded by a CMV minimal promoter (P_{minCMV}) and a tet operator (tetO). Only when doxycycline is present, rtTA binds to tetO and the ORF of interest is expressed in tissue A.

composed of the mutant version of the Tn10 tetracycline-resistance operon of *Escherichia coli* and a C-terminal portion of protein 16 of herpes simplex virus, which functions as a strong transcriptional activator. The rtTA is placed under the control of a tissue-specific promoter. Only in the presence of doxycycline, rtTA binds to the tet operator (tetO) that is placed on a separate construct and activates transcription from a minimal CMV promoter, which itself is inactive. This minimal promoter drives the expression of the desired gene. In summary, when doxycycline is added to the food, the transgene is expressed in a tissue-specific fashion. Conversely, it is also possible to shut down the expression of a transgene, using the tetracycline-controlled transactivator (tTA) system [2]. A refined system by which two genes can be simultaneously regulated in opposite directions is provided by a recent extension of this strategy [3]. More recently, a *lac* repressor transgene was developed which resembles a typical mammalian gene both in codon usage and structure and expresses functional levels of repressor in the mouse. This repressor was used to regulate the expression of a mammalian reporter-gene containing *lac* operator sequences. The *lac* repressor can repress the activity of a reporter-gene, which can subsequently be derepressed by the lactose analog IPTG [4].

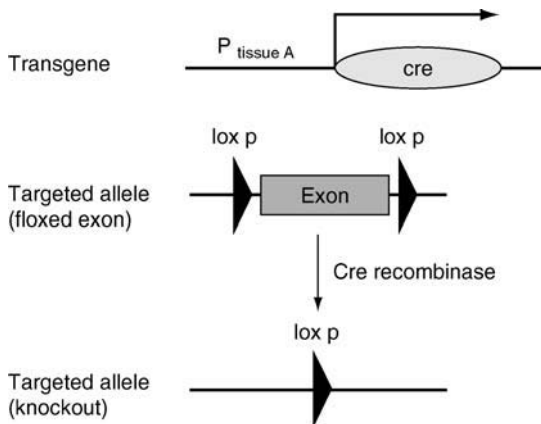
Tissue-Specific and Inducible Knockouts

Classical global knockouts may have a developmental or lethal phenotype and thus preclude the analysis of the phenotypic consequences of the lack of a gene in specific tissues in adult animals. With the development of the ►cre/loxP and ►flp/FRT systems, it has become possible to excise defined DNA fragments from the genome of specified cells. Cre and Flp are bacterial and yeast recombinases, respectively, which recognize loxP and FRT sequences, respectively. The most common

application is where the DNA fragment to be deleted is flanked by two parallel loxP or FRT sites. Expression of cre or flp, respectively, then leads to the excision or loss of the flanked fragment. This expression can be achieved in cell culture or in mice. In order to get rid of the neomycin resistance marker, this marker may be flanked by two FRT sites. Flp expression in embryonic stem cell culture or in mice expressing an appropriate flp transgene will eliminate the neo marker. In a typical example, essential exons of a gene are flanked by loxP sites (“floxed”) (Fig. 2). These loxP sites most likely do not have a functional activity on their own, so that the “floxed” mice can be considered functional wild type mice (which, however, may have to be confirmed in each case). Crossing the “floxed” mouse with a transgenic mouse carrying a *cre* transgene that is expressed in tissue A will result in some mice carrying both the targeted “floxed” allele and the *cre* transgene. Only in tissue A will the *cre* transgene be expressed and the exons flanked by loxP removed. In all other tissues, the loxP-flanked DNA fragment will be retained.

One major problem with this strategy is that the cre expression has to be strong enough and highly tissue- or even cell type-specific. Many *cre* transgenes currently in use have been constructed as standard transgenes using promoter elements of limited size via pronucleus injection, with the expression level and pattern being dependent on the integration site. Frequently, these transgenes lack the specificity of cre expression that

would be expected from the respective promoter that was used; in other words, the expression pattern of the *cre* transgene frequently does not match the expression pattern of the endogenous gene from which the promoter was isolated. It may be preferable to “knock-in” the *cre*-cDNA into the gene whose expression pattern one wants to replicate; potential disadvantages of this approach are that the cre expression via the endogenous promoter may be too weak for complete excision of flanked sequences and that it is experimentally the most time-consuming one. An alternative approach to achieve cre expression from endogenous promoters utilizes bacterial artificial chromosomes (BACs). BACs contain e.g., 150–200 kb genomic DNA fragments. BAC libraries spotted on nylon membranes are commercially available. Clones hybridizing to a probe of interest can then be purchased individually. Positive clones are mapped to determine the approximate position of the gene of interest in the BAC. A BAC can then be selected which contains the gene of interest roughly in the center and >50 kb of upstream sequence. Via homologous recombination in *E. coli*, the *cre* cDNA, can be introduced into the ATG start codon of the respective gene of interest. The BAC is then injected into a fertilized mouse egg and frequently integrated as a single copy. The integrity of the BAC ends can be confirmed e.g., by polymerase chain reaction. Though the integration occurs at a random position in the genome, it is hoped that the BAC contains all regulatory elements of the gene of interest [5]. In contrast to the “knock-in” approach, the BAC transgene typically does not change the function of endogenous genes. Other genes present in the BAC may be overexpressed. The BAC approach has the advantage of being usually much less time-consuming than the “knock-in” approach.



Transgenic Animal Models. Figure 2

Tissue-specific knockout using the cre/loxP system. To achieve a tissue-specific knockout, mice carrying a *cre* transgene (“Transgene”) and a targeted allele characterized by a “floxed” exon in the gene of interest are generated. In this example, the *cre* transgene is expressed under the control of a promoter that is expressed in tissues A ($P_{\text{tissue A}}$). The Cre protein is synthesized in tissue A, recognizes the two parallel loxP sites and excises the exon that is flanked by the two loxP sites. If the floxed and later deleted exon is essential for gene function, the mouse will display a tissue-specific gene knockout.

Transgenic and Knockout Mice in Functional Genomics Approaches

The linking of genomic sequence information to biological function is of major relevance for the identification of novel drug targets. Instead of analyzing the function of one gene at a time, entire sets of genes can be analyzed by multiplexing genes using “in vivo libraries.” In this approach, overlapping yeast artificial chromosomes (“YACs”) covering a specific chromosomal region, which has been implicated in a specific biological function, are introduced into the mouse germline. The resulting mouse lines will be analyzed with respect to the phenotype in question, which has to be sensitive to gene dosage. The presence or absence of the phenotype in various mouse lines permits functional mapping of the gene of interest. In another approach, cDNAs of unknown function can be overexpressed in mice. However, a gene inactivation approach may frequently reveal more about the normal function of genes than the overexpression of cDNAs.

ES Cell Libraries

In the private and the public sectors, projects are ongoing in which random mutations are generated in murine embryonic stem cells on a large scale (ES cell libraries). Tagged and presumably inactivated genes are easily identified by sequencing. These stem cells can be used to generate the respective knockout mice.

Pharmacological Relevance

Transgenic and gene-targeted animals have a significant impact in providing fundamental insights into biological systems and their pharmacological regulation. They provide valuable information, relevant for target discovery and validation, e.g., by clarifying the functional roles of potential drug targets or by generating animal models for human diseases that allow testing potential therapeutic strategies. Genetically modified animals may also be useful for the development of novel assays for toxicological testing. Tissue-specific and inducible gene expression greatly increases the selectivity with which these analyses can be performed.

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Transient Receptor Potential

The founding member of the TRP channel family, TRP, was identified as the product of a gene locus, which was referred to a transient receptor potential (TRP), because TRP mutant flies display a defect in light induced Ca^{2+} influx.

► TRP Channels

Transition State

A transition state is an unstable, high-energy configuration assumed by reactants in a chemical reaction on the way to making products. Enzymes can lower the activation energy required for a reaction by binding and stabilizing the transition state of the substrate.

Translation Inhibitors

► Ribosomal Protein Synthesis Inhibitors

Translocon

Multifunctional protein complex in the ER membrane. It translocates secretory proteins across and integrates membrane proteins into the ER membrane. The protein-conducting channel protein Sec61p is the most important component of the translocon.

► Protein Trafficking and Quality Control

Transmembrane Signaling

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Synonyms

Membrane signal transduction

Definition

Cellular functions are controlled by extracellular signals such as hormones, neurotransmitters, odorants, light and other chemical or physical stimuli. Only a few of these signal molecules, e.g., the highly lipid-soluble steroids or thyroid hormones, can diffuse across the

plasma membrane to interact with their intracellular receptors. Most regulatory factors are water-soluble and interact with membrane receptors to induce a signal transduction process that leads to the formation of intracellular signals or to the activation of and further integration within signaling cascades.

Basic Mechanisms

Transmembrane signaling processes involve the recognition and binding of an extracellular signal by an integral membrane receptor protein and the generation of intracellular signals by one or more effector proteins. Receptor and effector functions can be intramolecularly coupled by functional domains of the transmembrane protein or can be located on separate protein entities (intermolecular coupling). On the basis of intermolecular or intramolecular interactions of the signal-receiving receptor to the intracellularly located effector protein or protein domain, receptors can be divided into two groups.

Receptor Classification

Receptors that Couple to an Intramolecularly Linked Effector Domain

Receptors permanently linked to an effector consist of proteins with an extracellular ligand-binding receptor domain and a signal-generating effector domain (Fig. 1). Most of these receptors are composed of two to five structurally related or identical subunits. Effectors can be enzymes or ion channels whose activities are stimulated by agonist binding without significant delay.

1. *Enzyme-linked receptors* possess an extracellular domain with receptor function, a single transmembrane-spanning segment and an intracellular domain possessing catalytic activity. Ligand binding is typically decoded by receptor dimerization or by allosteric rearrangement of a preformed receptor oligomer. ▶ *Receptor tyrosine kinases*, which form the largest family of enzyme-linked receptors, bind various peptides such as growth factors or insulin and act, among others, through the canonical Ras/Raf/MEK/ERK class of ▶ *MAP kinase cascades*. Members of the ▶ *transforming growth factor β* (TGF β) receptor family bind TGF β , bone morphogenic proteins or activin, feature an intracellular serine/threonine kinase activity and act via SMADs [1]. The receptor-linked ▶ *guanylyl cyclases* are receptors for natriuretic peptides and guanylin. Their activation results in an increased intracellular formation of the second messenger ▶ *cGMP*. Some of these enzyme-linked receptors form homodimers, e.g., the receptor tyrosine kinases and the guanylyl cyclases. Receptor-type protein-tyrosine phosphatases (RTPs) exhibit adhesion protein-like extracellular domains, but their ligands

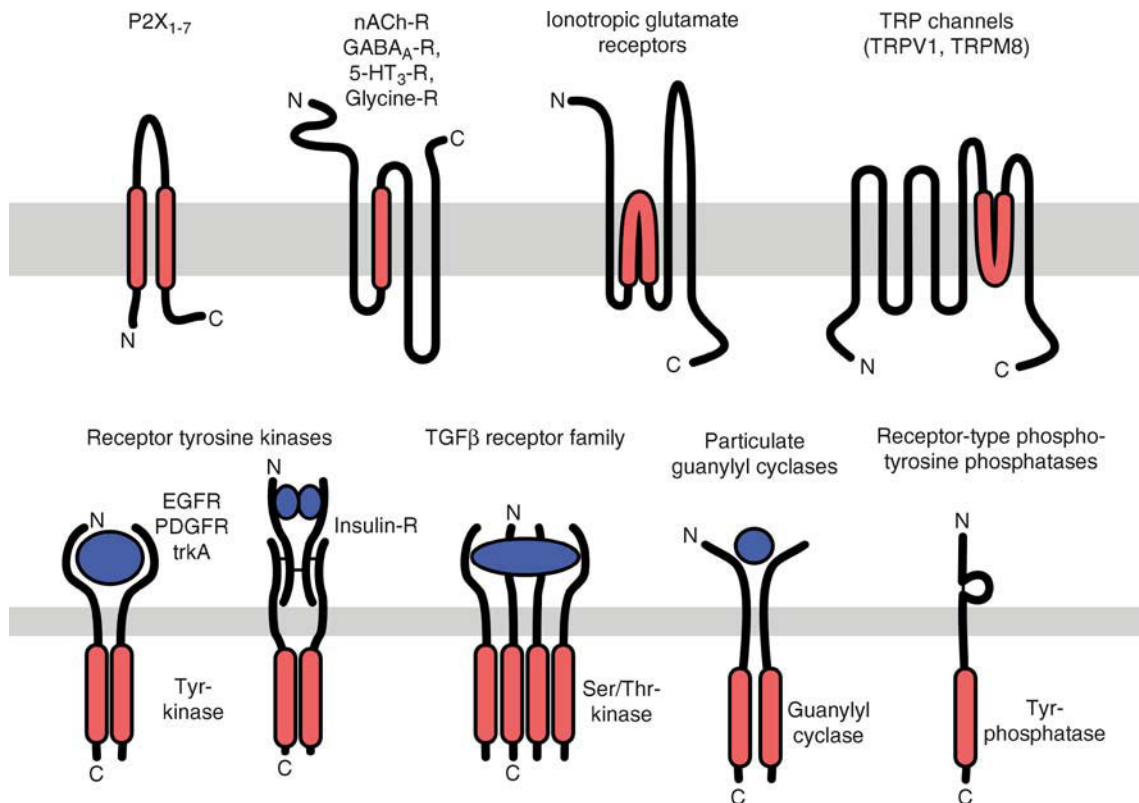
and specific downstream targets are only poorly defined.

2. ▶ *Ligand-gated ion channels* consist of several identical or related subunits which together form a central pore acting as the regulated effector. The ligand binding domain is typically located on or between the pore-forming subunits, but may also involve accessory subunits such as the sulfonyleurea receptor (SUR) subunit, which is associated with ▶ *ATP-dependent K^+ channels*. The ▶ *nicotinic acetylcholine receptors* and the 5-HT $_3$ ▶ *serotonin receptors* (both consisting of five structurally related subunits with four transmembrane domains each), ▶ *ionotropic glutamate receptors* (consisting of four structurally related subunits with three transmembrane domains each), some ▶ *transient receptor potential (TRP) channels* (consisting of four structurally related subunits with six transmembrane domains and a pore loop domain each) and the P2X ▶ *purinergic receptors* (consisting of three subunits with two transmembrane domains each) are members of this family; they are connected to a cation channel. On the other hand, ▶ *GABA $_A$ receptors* and glycine receptors (consisting of five structurally related subunits with four transmembrane domains each, similar to the nicotinic acetylcholine and the 5-HT $_3$ serotonin receptors) are members that are connected to anion channels.

Receptors Associated with an Effector System

Receptors that are intermolecularly coupled to an effector system consist of two or more protein components providing the ligand-binding site, transducer and effector functions (Fig. 2).

1. ▶ *G-protein-coupled receptors* (GPCRs, seven-transmembrane, 7TM or heptahelical receptors) interact with and activate regulatory heterotrimeric G-proteins and thereby regulate a variety of signal-generating enzymes and ion channels [2]. These receptors represent the largest family of receptor proteins. For about 270 of them, the ligands are known, another 140 are ▶ *orphan receptors* with unknown ligands, and about 900 are olfactory receptors (but two thirds of the human olfactory receptor genes are pseudogenes). Activated GPCRs interact with membrane-attached ▶ *heterotrimeric GTP-binding proteins* (G-proteins), consisting of one α -, β - and γ -subunit each. The β - and γ -subunits form a functional complex (G $\beta\gamma$), which is released from the GTP-bound α -subunit within the receptor-mediated activation process. G α_{GTP} and free G $\beta\gamma$ regulate the activity of effector proteins such as enzymes (e.g., adenylyl cyclases, phospholipases C β , phosphatidylinositol 3-kinases, guanine nucleotide exchange factors of Rho GTPases, and, in the retina, the cGMP ▶ *phosphodiesterase* type 6) and



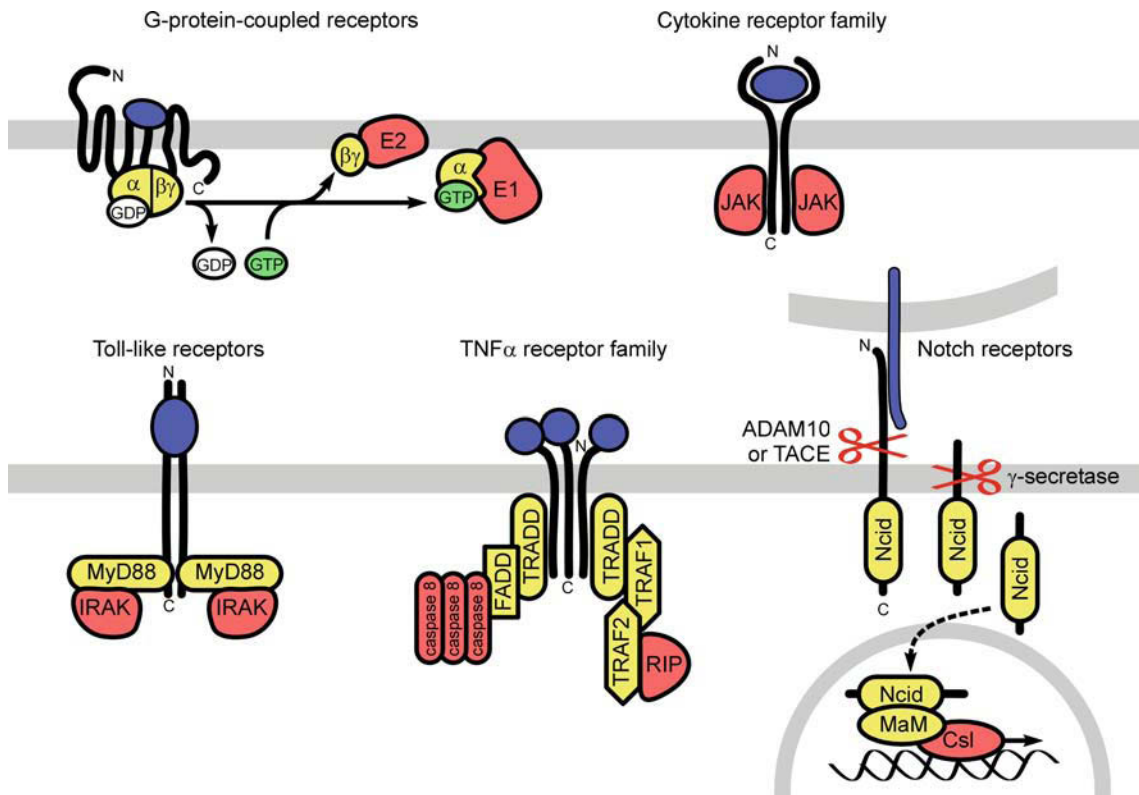
Transmembrane Signaling. Figure 1 Membrane topology of ligand-gated ion channels and of transmembrane receptors which are intramolecularly linked to an effector domain. Shown are single subunits of ligand-gated ion channel complexes, which are composed of three (P2X receptors), four (ionotropic glutamate receptors, TRP channels) or five (others) identical or structurally related subunits. Transmembrane segments or recursive loops aligning the ion-conducting pore are highlighted. Likewise, catalytically active cytosolic domains of enzyme-linked receptors are indicated (red).

ion channels (e.g., ►voltage-gated Ca^{2+} channels and ►inwardly rectifying K^{+} channels).

G-proteins are subdivided into four G-protein subfamilies according to structural similarities of their α subunits. The G_s subfamily (including G_s and G_{olf} proteins) is involved in ►adenylyl cyclase stimulation, whereas the activated $G\alpha$ and $G\beta\gamma$ subunits of the $G_{i/o}$ subfamily (including the G_i , G_o , G_z , transducin and gustducin isoforms) mediate adenylyl cyclase inhibition (G_i), cGMP ►phosphodiesterase type 6 stimulation (transducin), calcium channel inhibition ($G\beta\gamma$ from G_o) and potassium channel stimulation ($G\beta\gamma$ from G_i). Beside $G\beta\gamma$, members of the G_q subfamily (largely G_q and G_{11} , but in some cellular systems also G_{14} and $G_{15/16}$) by their activated α -subunits cause activation of phospholipase $\text{C}\alpha$ isoforms, thereby inducing a ►PI response. The α subunits of the G_{12} subfamily, consisting of G_{12} and G_{13} , can stimulate guanine nucleotide exchange factors (GEFs) of Rho, thereby mediating ►cytoskeletal rearrangements and, via Rho-dependent kinase (ROCK), Ca^{2+} -independent cellular contraction, e.g., of ►vascular smooth muscle.

Most GPCRs interact with and activate more than one G-protein subfamily, e.g., with G_s plus $G_{q/11}$ (histamine H_2 , parathyroid hormone and calcitonin receptors), G_s plus G_i (luteinising hormone receptor, β_2 -adrenoceptor) or $G_{q/11}$ plus $G_{12/13}$ (thromboxane A_2 , angiotensin AT_1 , ►endothelin ET_A receptors). Some receptors show even broader G-protein coupling, e.g., to G_i , $G_{q/11}$ plus $G_{12/13}$ (►protease-activated receptors, lysophosphatidate and sphingosine-1-phosphate receptors) or even to all four G-protein subfamilies (thyrotropin receptor). This multiple coupling results in multiple signaling via different pathways and in a concerted reaction of the cell to the stimulus.

2. ►Cytokine receptors that couple to the ►JAK-STAT Pathway decode the signaling through hematopoietic cytokines (erythropoietin, thrombopoietin, colony-stimulating factors), prolactin, growth hormone, the α -, β - and γ -►interferons, and a number of immunomodulatory interleukins [3]. They form homodimeric or heterodimeric receptor complexes, which after ligand binding recruit and activate isoforms of Janus kinases (JAKs). Activated JAKs in turn



Transmembrane Signaling. Figure 2 Membrane topology of receptors that are associated with effector proteins. Upon binding to their cognate ligands (cyan), receptor proteins without intramolecularly linked effector domain couple via transducer proteins (yellow) to or directly recruit and activate effector proteins (red). Notch receptors release their transducer domains upon proteolytic cleavage. α , β and γ stand for G-protein α -, β - and γ -subunits, respectively.

phosphorylate a subset of STAT proteins. Phosphorylated STAT proteins dimerize and translocate to the nucleus where they act as transcription factors. Other receptors that recruit nonreceptor tyrosine kinases to exert downstream signaling include the B cell receptor, the T cell receptor and the Fc ϵ receptor.

3. **TNF receptors** constitute a family of transmembrane receptors that share homology with the tumor necrosis factor α (TNF α) receptor and contain a highly conserved stretch of 80 amino acids that has been coined the death domain. Ligands that bind to and activate receptors of the TNF receptor superfamily include several cytokines, inducers of apoptosis and lymphotoxins. Upon ligand binding, TNF receptor-related proteins oligomerize and recruit TNF receptor-associated death domain protein (TRADD), Fas-associated death domain proteins (FADD), TNF receptor-associated factors (TRAFs) and receptor-interacting protein (RIP), a serine-threonine kinase. Downstream signaling pathways of TNF receptors include the initiation of the caspase 8-dependent proapoptotic pathway, the regulation of gene transcription via the IKK/I κ B/NF- κ B pathway, leading to a disinhibition and nuclear translocation

of **nuclear factor- κ B** and the activation of the JNK and p38 classes of **MAP kinase cascades**.

4. **Toll-Like receptors** (TLR) and the interleukin-1 receptors (IL-1R) constitute a superfamily of cell surface receptors that mediate the recognition of specific patterns of pathogens and thereby play a key function in triggering adaptive immune responses. The TLR isoforms bind bacterial membrane components such as lipopolysaccharides, lipoproteins and peptidoglycans, but also double-stranded viral RNAs or unmethylated CpG motifs, which are especially abundant in prokaryotic genomes. The IL-1 family of IL-1R ligands comprises IL-1 α , IL-1 β , IL-18 and IL-1RA. Intracellular responses that are triggered by TLRs and interleukin-1 receptors are initiated by formation of a complex between the respective receptor, a TIR domain-bearing adaptor protein (e.g., MyD88) and IL-1R-associated serine/threonine kinases (IRAKs). Via binding and phosphorylation of TRAF proteins, the IRAK-mediated signaling feeds into the IKK/I κ B/NF- κ B and JNK/p38 pathways.
5. **Notch receptors** are especially important during developmental stages by mediating lateral inhibition,

lineage decision and cellular segregation. The Notch family of cell surface receptors shares an architecture with an extracellular domain consisting of multiple EGF-like repeats, a single transmembrane-spanning segment and an ankyrin repeat-bearing Notch intracellular domain (Nidc). Binding of the ligands Delta or Jagged to Notch receptors triggers sequential proteolysis involving extracellular shedding by ADAM-type metalloproteases and subsequent intramembrane cleavage by γ -secretase, resulting in the release of the Notch intracellular domain (Nidc) into the cytosol. Nuclear targeting of Nidc and interaction with coactivators (Mastermind 1–3) or the corepressor SMRT together with DNA-binding proteins of the Csl family mediate regulation of gene transcription.

6. **Cell adhesion molecules** such as integrins, cadherins, claudins and selectins do not only act as transmembrane proteins involved in cell–cell or cell–matrix interactions but are also involved in signal transduction processes (e.g., the collagen-induced platelet adhesion and aggregation). Integrins even mediate inside-out signaling by adopting inactive and active conformations.

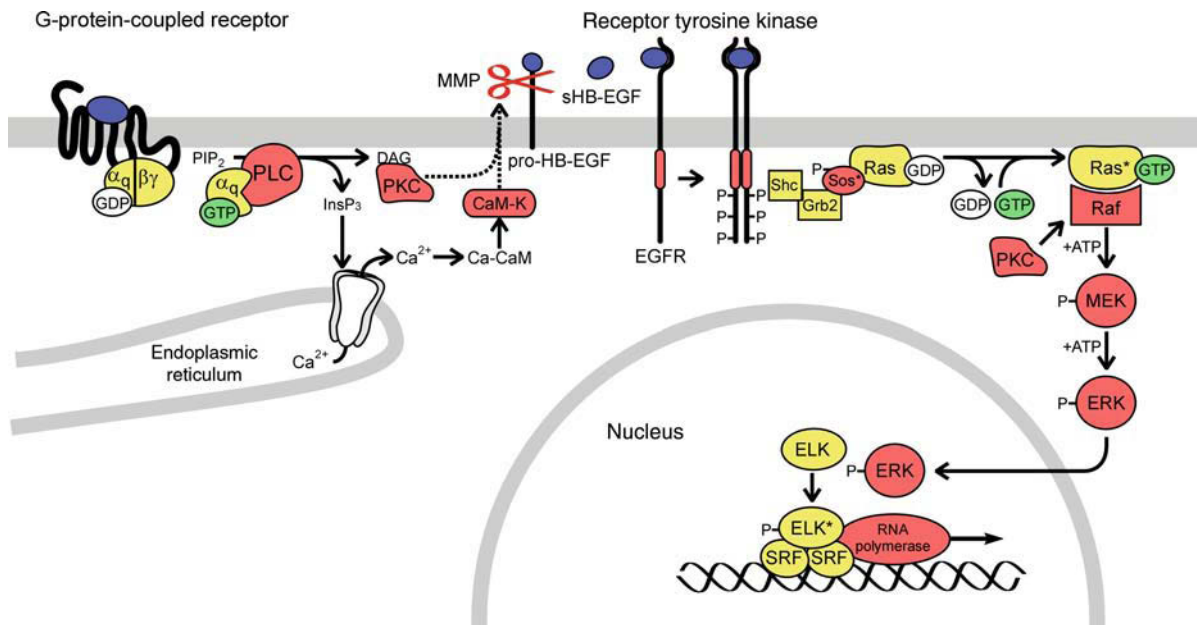
Besides classical receptor classes that bind endogenous or naturally occurring ligands and act via prototypical intracellular signaling cascades, a considerable number of transmembrane proteins are referred to as “receptors” albeit their natural ligands are unknown or they do not

initiate a characteristic downstream signaling cascade. These proteins include low density lipoprotein (LDL)-like receptors, the transferrin receptor, receptors for toxins or viruses as well as some target proteins for nonnatural chemical ligands (e.g., the dihydropyridine receptors, the sulfonylurea receptor).

Receptor Transactivation

Transmembrane signaling by one type of receptor may affect signaling pathways that are primarily used by other receptor types. Transactivation of receptor tyrosine kinases downstream of G-protein-coupled receptors is a common phenomenon leading to the activation of the Ras/Raf/MEK/ERK cascade and nuclear transcription events downstream of G-protein signaling. Receptor transactivation means that the signaling of one receptor class cannot be seen as an event separate from other regulations but has to be considered as part of a signaling network within the cell. Long-term changes of cellular responses and tissue functions in response to rapidly and transiently acting agonists may, therefore, result from transactivation of receptor classes that are coupled to transcriptional regulation.

A specific form of transmembrane signaling is given by receptor tyrosine kinase transactivation via the triple membrane-spanning (TMS) pathway [4] (Fig. 3). A GPCR ligand-induced shedding of membrane-bound growth hormone precursors such as pro-EGF, pro-HB-EGF, pro-Epiregulin or pro-Amphiregulin has been



Transmembrane Signaling. Figure 3 Triple membrane-spanning pathway. Control of growth and differentiation downstream of G-protein-coupled receptors often involves transactivation of receptor tyrosine kinases to induce activation of the canonical Ras/Raf/MEK/ERK pathway, nuclear translocation of activated ERK1/2 and subsequent regulation of gene transcription. Cyan: receptor ligands; yellow: transducer proteins; red: catalytically active effectors; green: active cofactors.

recognized to form a variant of transmembrane signaling, in which the signal enters the cell through GPCR activation, exits the cell again by an as yet unresolved mechanism to secrete or activate matrix metalloproteinases and reenters the cell by the autocrine or paracrine action of released mature growth factors that bind to and activate their cognate receptor tyrosine kinases. Besides direct actions of GPCR effectors and intracellular transactivation modules, the TMS pathway appears to be an important mechanism to mediate mitogenic or comitogenic responses to GPCR stimulation.

Quantitative Aspects

The number of receptor molecules is typically restricted to a few thousands per cell, and ligands are in most cases bound with nano- to low micromolar affinities. The effectors coupled to a receptor, independently of being an enzyme or ion channel, amplify the signal either by their catalytic activity or by allowing thousands of ions to flow through an activated ion-conducting pore. In G-protein-coupled receptors, signal amplification is accomplished by the fact that a single active receptor can mediate GDP/GTP exchange of multiple G-proteins. Activated receptor tyrosine kinases typically expose several phosphotyrosines and thereby recruit more than one SH2 domain-bearing adapter or downstream signaling protein. By using whole cascades of transducer molecules and enzymes or by expressing additional amplifying systems, specialized cells are capable of recognizing a very low number of agonist molecules and convert even tiny input signals into robust cellular events such as exocytosis, depolarization or transcriptional regulation.

Spatial Aspects

To ensure a tight control of signaling strength and fidelity, to accelerate the signal propagation or to restrict signaling processes to certain cellular compartments, some transmembrane signaling pathways are highly organized and physically concentrated in higher order molecular networks. Such clustered structures have also been referred to as transducisomes or signalplexes. Higher order signaling complexes can be formed by means of scaffolding proteins such as PDZ domain-bearing proteins or ►AKAPs, by segregating into membrane subdomains including caveolae or rafts, or by clustering within cellular protrusions (e.g., focal adhesions, filopodia, lamellipodia, membrane ruffles, axonal growth cones or primary cilia). Often, disruption of the respective clustering mechanism severely disturbs transmembrane signaling.

Temporal Aspects

Ligand-induced signal transduction processes that are mediated by a ligand-operated ion channel, e.g., by the nicotinic receptor in muscle, result in an almost

immediate (msec scale) cellular response as the number of components is very small and no enzymatic step is involved. In comparison, the onset of cellular responses to ligands interacting with GPCRs and inducing the formation of intracellular signals that stimulate protein phosphorylation is slower (sec). Cellular responses induced by growth factors or cytokines acting via phosphorylation cascades on transcription factors typically occurs after several minutes. Receptor classes that couple to gene transcription events and subsequent changes of protein expression typically exhibit lag times of 30 min to several hours until the cellular response can be seen.

Pharmacological Intervention

The naturally occurring ligands of membrane receptors, i.e., hormones, neurotransmitters, growth factors and cytokines, act as ►agonists, i.e., upon binding they induce the generation of intracellular signals leading to a cellular response [5]. Partial agonists are compounds that activate a certain receptor, but with less than the full intrinsic activity of the natural agonist. For receptors coupling to more than one effector molecule and signaling cascade, the development of pathway-selective agonists may become possible. ►Antagonists that act as blockers of the agonist effects have been developed as drugs.

The pharmaceutical industry has developed drugs that act as ►inverse agonists, i.e., these compounds through the same receptor cause opposite cellular reactions. Agonists are supposed to shift the equilibrium between the inactive and active (effector-coupled) forms towards the latter one, inverse agonists do the opposite; they can be used to silence a constitutively active receptor. Histamine H₂, H₃ and H₄ receptors are examples for receptors with high constitutive activity, and H₂ blockers such as cimetidine and ranitidine actually are powerful inverse agonists. Similarly, naloxone acts as an inverse agonist at -opioid receptors.

Most hormones and neurotransmitters act via more than one ►receptor subtype. Receptor subtypes often act on different effectors, thereby allowing complex, and in some cases opposite responses of certain tissues to a systemically distributed hormonal regulator (e.g., adrenaline-mediated vasodilatation via β_2 adrenoceptors and vasoconstriction via α_1 adrenoceptors). Receptor subtypes can show differences in agonist and antagonist binding, a property that can be employed to achieve cell- or tissue-specific action of synthetic subtype-specific agonists or antagonists. Accordingly, the pharmaceutical industry has developed numerous compounds discriminating between receptor subtypes, thus providing higher selectivity for certain receptors than the naturally occurring agonists. Among neurotransmitter receptors, the coexistence of ►ionotropic receptors (linked to ligand-gated ion channels) and of ►metabotropic receptors (i.e., G-protein-coupled

receptors) is most striking, i.e., ►[nicotinic](#) versus ►[muscarinic acetylcholine receptors](#), 5-HT₃ versus the other ►[serotonin receptors](#), ►[GABA_A](#) versus ►[GABA_B](#) receptors and ►[P2X](#) versus ►[P2Y](#) purinergic receptors.

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Transport ATPase

► [Table appendix: Membrane Transport Proteins](#)

Transporter

► [Table appendix: Membrane Transport Proteins](#)

Transposon

Transposons are mobile DNA elements (sizes ~2.5–23 kbp) that move from one place to another in the chromosome or onto extrachromosomal genetic elements within the same cell. They are flanked by inverted repeats at their ends and encode among other proteins a transposase that is needed for the transposition process. Resistance genes in the transposon are often parts of integrons. These are structures that carry an integrase responsible for the insertion of the resistance gene cassettes into the integron.

► [Microbial Resistance to Drugs](#)

Transverse Tubule

Synonyms

T-Tubule

Definition

T-tubule is a transverse invagination of the plasma membrane, which occurs at the specified sites characteristic to animal species and organs, i.e. at the Z-line in cardiac ventricle muscle and non-mammalian vertebrate skeletal muscle and at the A-I junction in mammalian skeletal muscle. It is absent in all avian cardiac cells, all cardiac conduction cells, many mammalian atrial cells and most smooth muscle cells. T-tubule serves as an inward conduit for the action potential.

► [Ryanodine Receptor](#)

Triazenes

Triazenes are compounds having the structure RN=N–NR₂ (not all R=H, and one R commonly aryl).

► [Alkylating Agents](#)

Trichomonas Vaginalis

Trichomonas vaginalis is a ubiquitous sexually transmitted anaerobic flagellate causing vaginitis in women and prostate gland infection in men.

► [Antiprotozoal Drugs](#)

Tricyclic Antidepressants

Tricyclic antidepressants are an important group of antidepressants. They block the uptake of monoamines by nerve terminals by competing for the binding site of the reuptake carrier protein.

► [Antidepressant Drugs](#)

Triiodothyronine

Triiodothyronine (3',5,3-L-triiodothyronine, T₃) is a thyroid hormone. It is produced by outer ring deiodination of thyroxine (T₄) in peripheral tissues. The biologic activity of T₃ is 3–8 times higher than that of T₄. T₃ is 99.7% protein-bound and is effective in its free non-protein-bound form. The half-life of triiodothyronine is about 19 h. The daily turnover of T₃ is 75%. Triiodothyronine acts via nuclear receptor binding with subsequent induction of protein synthesis. Effects of thyroid hormones are apparent in almost all organ systems. They include effects on the basal metabolic rate and the metabolisms of proteins, lipids and carbohydrates.

► Antithyroid Drugs

Tropism

A viral tropism is the specificity of a virus for particular host tissues and cells.

► Gene Therapy

Troponin

Troponin specifically regulates muscle contraction.

► Ca²⁺-Binding Proteins

3',5,3-L-Triiodothyronine

► Triiodothyronine

Trimodal Distribution

A trimodal distribution is a frequency distribution of a certain phenotype with three peaks separated by two antinodes.

► Pharmacogenetics

Tripeptidyl-peptidase

An exopeptidase that sequentially releases a tripeptide from the N-terminus of a protein or peptide. Tripeptidyl-peptidases are included in Enzyme Nomenclature sub-subclass 3.4.14 along with dipeptidyl-peptidases.

► Non-viral Peptidases

TRP Channels

VEIT FLOCKERZI

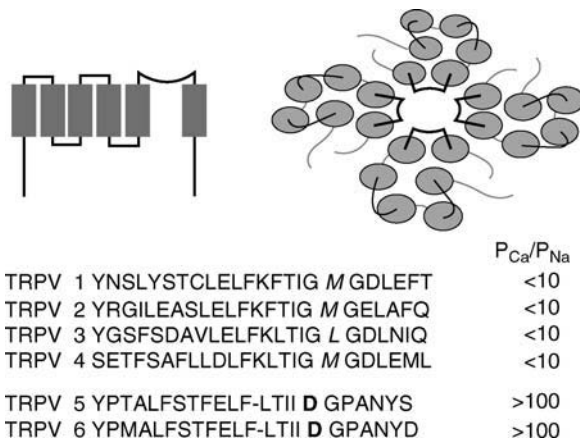
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Definition

A superfamily of cation channels conserved in mammals, flies, worms and yeast. The various TRP-proteins bear sequence and predicted structural similarities to the founding member of this superfamily, ► [transient receptor potential](#) (TRP), a light activated cation channel in the *Drosophila* photoreceptor.

Basic Characteristics

So far, more than 20 TRP genes have been identified in mammals [1, 2]. Almost all TRPs are supposed to form ion channels that are widely expressed in the nervous system, and which may be the primary site of Ca²⁺ entry in nonexcitable cells. A TRP protein contains six segments, predicted to cross the cell membrane, and a putative pore loop within the extracellular linker separating the fifth and sixth transmembrane segments (Fig. 1, top left), but lacks the voltage-sensing element (S4) present in voltage-gated channels. Almost all TRPs are activated by as yet unclear mechanisms (see below) involving phospholipase C (PLC) and phosphatidylinositol pathways. Four or five TRP proteins form homooligomeric and heterooligomeric channels (Fig. 1, top right) and evidence is steadily emerging that these



TRP Channels. Figure 1 TRP protein topology and pore loops of the members of the TRPV subfamily. Top, left: TRP proteins comprise six predicted transmembrane segments linked by extracellular and cytosolic protein domains. The pore loop resides within the extracellular linker separating the fifth and sixth transmembrane segments. Top, right: View perpendicular to the surface of the plasma membrane. Four TRP proteins assemble to form an ion channel. The four pore loops line the ion permeant pathway. Circles represent transmembrane segments. Bottom: Amino acid sequence alignment of the pore loops of the members of the TRPV subfamily. The D shown in bold represents the negatively charged aspartic acid residue responsible for the high Ca^{2+} selectivity of channels formed by TRPV5 and, presumably, TRPV6. P_{Ca} and P_{Na} , relative permeability to Ca^{2+} (P_{Ca}) and to Na^{+} (P_{Na}).

channels are integrated into signal transduction complexes by ►scaffolding proteins. Based on their structural similarities the TRP proteins fall into three subfamilies of channels, TRPC, TRPV and TRPM (Table 1). Two additional subfamilies, TRPP and TRPML, which are more distantly related to TRP, include ►PKD2 and ►mucolipin.

Ion Channel Properties

Essential molecular determinants of ion permeation through TRP channels should reside within a protein domain, which participates in the formation of the ion permeable pathway of the channel. This domain is therefore called the pore loop. So far this region has only been characterized for TRP channels formed by members of the TRPV group. Here, ►TRPV1, TRPV2, TRPV3 and ►TRPV4 channels do only poorly discriminate between monovalent and divalent cations, especially Na^{+} and Ca^{2+} , and accordingly, are called “nonselective” cation channels. In contrast, ►TRPV5 and ►TRPV6 form highly selective Ca^{2+} channels. Thus the relative permeability to Ca^{2+} (P_{Ca}) is more than 100-fold higher than to Na^{+} (P_{Na}) in these two channels. A single negatively charged aspartic acid residue

TRP Channels. Table 1 The superfamily of mammalian TRP channels

TRPC subfamily	TRPV subfamily	TRPM subfamily
TRPC1	TRPV1	TRPM1
TRPC2	TRPV2	TRPM2
TRPC3	TRPV3	TRPM3
TRPC4	TRPV4	TRPM4
TRPC5	TRPV5	TRPM5
TRPC6	TRPV6	TRPM6
TRPC7		TRPM7
		TRPM8

(D, shown in bold in Fig. 1 bottom) in the pore loop of TRPV5, which is conserved in TRPV6, but not in the other members of the TRPV subfamily, has been shown to be responsible for the high Ca^{2+} selectivity of channels formed by TRPV5 [3].

For all other members of the TRP family it still has to be shown whether the presumptive pore loop or other protein domains actually line the ion conducting pathway of the channel. Based on the results showing that expression of most TRPC channels yields currents carried by Na^{+} and Ca^{2+} , and that expression of ►TRPM4 and ►TRPM7 channels yields currents carried by Na^{+} but not by Ca^{2+} (TRPM4) or even currents carried by Mg^{2+} or Ca^{2+} (TRPM7), it seems likely that the pore structures of these channel proteins vary considerably.

Modes of Activation

TRP channels vary significantly in their modes of activation [4]. Given that *Drosophila* TRP requires PLC for activity *in vivo*, mammalian TRPs were predicted to be PLC-dependent ion channels. Activation of PLC could be coupled to TRP channel activation via relief of phosphatidylinositol-4,5-bisphosphate (PIP_2)-mediated channel repression and/or production of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). According to one mechanism, referred to as ►store-operated Ca^{2+} entry, transient IP_3 induced release of Ca^{2+} from intracellular stores induces sustained Ca^{2+} influx by activation of a plasma membrane Ca^{2+} entry channel. Leading contenders for channels activated by the latter mechanism are TRPC1, TRPC3, TRPC4 and TRPC5, although none of the published reports have unequivocal evidence for such a mechanism. In addition, members of the TRPC subfamily could be activated by diacylglycerol (TRPC3, TRPC6), by direct interaction with the IP_3 receptor (TRPC3), and – like TRPV1 and TRPM7 – by relief of (PIP_2)-mediated repression of the channel, to mention a few.

Members of the TRPV subfamily are activated by a broad range of stimuli including heat (TRPV1, TRPV2, TRPV3 and TRPV4), ligands such as ►capsaicin

(TRPV1) and ►endocannabinoids (TRPV1, TRPV4, see below), osmolarity and cell volume (TRPV4), hydrolysis of PIP₂ and protons (TRPV1). So far, TRPV5 and TRPV6 channels are unique in that they represent the only highly Ca²⁺-selective channels within the TRP superfamily (see above) and are activated by low intracellular Ca²⁺.

Three TRPM proteins, ►TRPM2, TRPM6 and TRPM7, are distinguished from other TRPs and other known ion channels, in that they consist of enzyme domains linked to the C termini of the ion channel domains resulting in “Chanzymes” TRPM6 and TRPM7 encode TRP proteins linked to functional atypical protein kinases; however, at least TRPM7 channel activity does not require the kinase domain for activation [5]. Currents through channels formed by TRPM6, TRPM5, TRPM1 and TRPM3 have not been identified electrophysiologically so far. Currents through TRPM2 channels are activated by ADP-ribose and changes in the redox status within the cell. TRPM4 is a Ca²⁺-activated channel (EC₅₀~0.3–0.4 μM), nonpermeant for Ca²⁺, whereas ►TRPM8 can be activated by a drop in temperature below 26°C or by agents, such as menthol and icilin, that evoke a cool sensation.

Pharmacological Relevance

A clear limitation of studies on TRP channels is the lack of specific channel blockers. Organic compounds (e.g., ruthenium red, econazole, miconazole, SK&F96365) and anorganic blockers (e.g., La³⁺, Gd³⁺) have generally found to be of insufficient potency and specificity. The few exceptions include compounds such as capsaicin (EC₅₀~0.7 μM) and ►resiniferatoxin (EC₅₀~0.04 μM) as activators and capsazepine (IC₅₀~0.3 μM) as blockers of the TRPV1 currents, the endogenous cannabinoid receptor agonist anandamide (EC₅₀~4.9 μM) and related endocannabinoids as activators of TRPV1 and TRPV4 currents and 4α-PDD, a phorbol derivative, as activator of TRPV4 currents (EC₅₀~0.2 μM).

Biological Relevance and Emerging Roles for Mammalian TRP Channels

Most of the TRP channels have only been characterized in recombinant systems but not as functional channels in a physiological context; still others have not even been characterized as functional channels. Firm data on their biological relevance will emerge basically by the combination of three routes: (i) By the development and identification of toxins and agents that can be used to specifically block currents through a given TRP channel in primary cells and tissues (see above “Pharmacology”). (ii) By linking diseases to mutations in TRP genes; e.g., familial hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, implicating a role for TRPM6 in renal Mg²⁺ uptake. Other examples are mucopolidosis type

IV, a neurodegenerative disease caused by disruption of the mucopolin gene, a TRP of the TRPML subfamily, and common causes of polycystic kidney disease are mutations within PKD2, a TRP of the TRPP subfamily. (iii) By transgenic mice with targeted disruption of single TRP genes or a combination of TRP genes; so far three TRP-deficient mice have been described that underline the biological roles of TRPC2, TRPC4 and TRPV1 in social and sexual behavior (TRPC2), vasorelaxation and microvascular endothelial permeability (TRPC4) as well as nociception and thermal hyperalgesia (TRPV1). There are suggestions that a number of TRP-related proteins may also have roles in growth control, and changes in the expression of these channels may contribute to certain forms of cancer. A decrease in expression of TRPM1 appears to be a prognostic marker for metastasis in patients with localized malignant melanoma. Alterations of TRPM5 may be associated with Beckwith–Wiedemann syndrome, a predisposition to a variety of neoplasms, whereas expression of TRPM8 and TRPV6 appears to be upregulated in prostate cancers; these two TRPs may represent prognostic markers for prostate cancer and targets for new drugs to treat this disease.

- Table Appendix: Membrane Transport Proteins
- Non-selective Ca²⁺ Channels
- Voltage-dependent Ca²⁺ Channels
- Smooth Muscle Tone Regulation

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TRPM4

TRPM4 also known as the Ca²⁺-activated nonselective cation (CAN-) channel, mediates cell membrane depolarization in many excitable and nonexcitable cells.

- TRP Channels

TRPM7

TRPM7, also known as mTRP-PLIK, ChaK1 or LTRPC7, is a Mg^{2+} -permeable, nonselective cation channel, expressed in kidney, placenta, heart and seemingly, every cell examined, including blood cells and cell lines commonly used for expression studies. The monovalent current is inhibited by Mg^{2+} ($IC_{50} \approx 0.6$ mM) – hence MagNum (magnesium- and nucleotide-regulated metal current) or MIC (Mg^{2+} -inhibited current) – whereas activation involves reduction of local phosphatidylinositol 4,5-bisphosphate (PIP2) concentrations. TRPM7 has been identified as a protein interacting with phospholipase C β 1 (PLC β 1) and has, like TRPM6, the unique feature of being an ion channel and a protein kinase, although TRPM7 channel activity appears not to require the kinase domain for activation.

► TRP Channels

TRPM8

TRPM8 is also known as TRP-P8 or the cold receptor.

► TRP Channels

TRPV1

TRPV1, also known as the capsaicin- or vanilloid-receptor, is a nonselective cation channel expressed e.g., in neurons of the dorsal root and trigeminal ganglions, which integrates multiple pain-producing stimuli including heat, protons, capsaicin, and resiniferatoxin. In addition, TRPV1 currents can be activated by anandamide, protein kinase C (PKC), and by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2).

► TRP Channels

► Analgesics

TRPV4

TRPV4, also known as TRP12, OTRPC4 or VROAC, is a nonselective cation channel, predominantly

expressed in endothelium, kidney, heart and brain. It is activated by a decrease in extracellular osmolarity, by cell swelling, arachidonic acid, anandamide, and by 4 α -PDD, a phorbol derivative.

► TRP Channels

TRPV5 and TRPV6

TRPV5 and TRPV6, also known as the epithelial Ca^{2+} channel or ECaC (TRPV5) and Ca^{2+} transporter 1 or Ca^{2+} transporter-like (TRPV6), are the only two Ca^{2+} -selective TRP channels identified so far. They may function in vitamin D-dependent transcellular transport of Ca^{2+} in kidney, intestine and placenta. TRPV6 is also expressed in pancreatic acinar cells, and in prostate cancer, but not in healthy prostate or in benign prostate hyperplasia.

► TRP Channels

True ChE

► Cholinesterases

Trypsin

Trypsin is a major proteolytic digestive enzyme and the identified endogenous ligand for proteinase-activated receptor 2 (PAR 2).

► Proteinase-activated Receptors

Trypsin-like Proteinases

Trypsin-like proteinases are serine proteinases that recognized peptide residues with positively charged side chains (arginyl or lysyl residues) and that effect

hydrolysis of the polypeptide chain on the carboxy-terminal side of these residues. All clotting and complement cascade proteinases are trypsin-like.

► Non-viral peptidases

TSH Receptor

Thyrotropin (TSH) regulates the production and secretion of thyroid hormones as well as thyroid epithelial cell growth via the TSH receptor. The TSH receptor belongs to the family of G protein-coupled receptors. It is composed of 764 amino acids. The receptor contains a long hydrophilic region orientated towards the exterior of the cell (ectodomain), 7 hydrophobic transmembrane domains and a short cytoplasmic region.

► Antithyroid Drugs

T-tubule

A T-tubule is a transverse invagination of the sarcolemma, which occurs at characteristic sites in animal species and organs, i.e. at the Z-membrane in cardiac ventricle muscle and non-mammalian vertebrate skeletal muscle and at the A-I junction in mammalian skeletal muscle. It is absent in all avian cardiac cells, all cardiac conduction cells, many mammalian atrial cells and most smooth muscle cells. It serves as an inward conduit for the action potential. The surface area in the skeletal muscle can reach 6–8 times that of a cylinder with the same radius. In the T-tubule, Na-channel, Ca-channel and other important channels and transporters can be detected.

► Ryanodine Receptor

TTX

► Tetrodotoxin

Tubulin

Tubulin is a major component of the cellular cytoskeleton. Tubulin polymers (microtubules) are important for cell division (mitotic spindle) and the chemotaxis and phagocytosis of neutrophils. Prevention of tubulin polymerisation by colchicine accounts for the therapeutic effects of this drug in acute gouty arthritis and its anti-mitotic effects.

► Anti-gout Drugs

► Cytoskeleton

α and β Tubulin

α and β tubulins associate into heterodimers to form microtubules. Both tubulins have molecular weights of about 50 kD and bind GTP. In higher eukaryotes, there are up to 7 isoforms of α and β tubulin, respectively, which are encoded by different genes.

► Cytoskeleton

Tumor Necrosis Factor

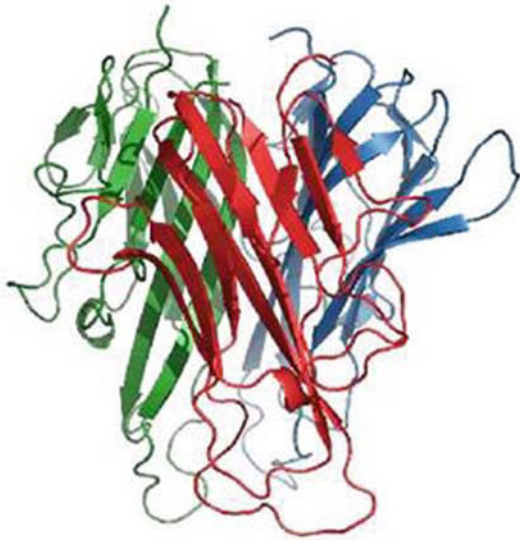
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Synonyms

TNF- α ; Cachectin; Cachexin

Definition

Human tumor necrosis factor (TNF) (Fig. 1) is a hormone-like proinflammatory peptide belonging to the group of ►cytokines. It is mainly produced by cells of the immune system in response to infection, ►inflammation, or cell damage. Dysregulated TNF is an important factor in many pathological situations, like sepsis, rheumatoid arthritis, inflammatory bowel disease (Crohn's disease), and Cachexia. The cytotoxic activity of TNF is of interest in development of new antitumoral strategies.



Tumor Necrosis Factor. Figure 1 Homotrimeric structure of soluble TNF (51kDa). TNF monomers (17kDa) in individual subunits fold into a jelly roll pattern that is composed of anti-parallel β strands separated by intervening loop regions, which are represented by green, red, or blue color.

Basic Characteristics

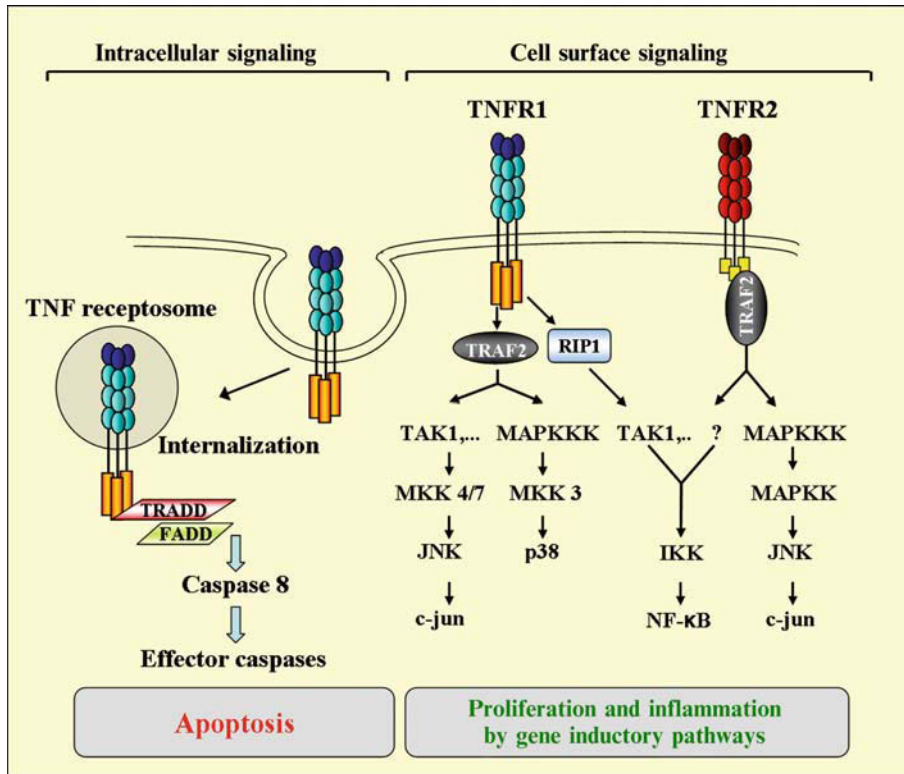
The antitumoral effects of TNF had been first noted in the late nineteenth century by W.B. Coley, a New York surgeon. Coley realized that bacterial infections can cause an antitumoral response in cancer patients. Consequently, he produced preparations of Gram-negative bacteria to treat patients with tumors. About 80 years later, a single, inducible serum protein was discovered that can induce hemorrhagic necrosis of tumors. TNF was molecularly cloned in 1984. Today, TNF is considered as the representative member of a large family of **cytokines** consisting of 19 identified ligands so far [1]. TNF exerts fundamental effects on cell proliferation and death, *inflammation*, and immunological and neuronal cell functions.

TNF is expressed as a 26 kDa **type II transmembrane protein** of 233 amino acids. A soluble form of TNF is generated by proteolytic cleavage close to the transmembrane region releasing the 17 kDa C-terminal extracellular region of the molecule consisting of 157 amino acids. **Metalloproteinases** like TACE (TNF α converting enzyme) convert the membrane bound form to the soluble form of TNF. Both forms confer bioactivity as self-assembled, noncovalently bound homotrimers (Fig. 1) interacting at hydrophobic interfaces. TNF binds to two cellular membrane receptors, TNFR1 (CD120a, TNFR55) and TNFR2 (CD120b, TNFR80) [2].

Both tumor necrosis factor receptors (TNFR) are **type I transmembrane proteins**, also belonging to a large family of proteins, the TNFR superfamily

comprising 29 receptors up to now [1]. TNFR1 and TNFR2 are characterized by four well-conserved cysteine-rich domains in their extracellular parts, a hallmark of the whole TNFR superfamily. Intracellularly, the TNF receptors are distinct. TNFR1 carries a so-called **death domain** that is shared by seven other members of the TNFR superfamily, that is, Fas, DR3 (TRAMP), DR4 (TRAILR1), DR5 (TRAILR2), DR6, EDAR, and NGFR, whereas TNFR2 contains a TNFR-associated factor (TRAF) binding domain, marking also most of the residual members of the TNFR superfamily. Although the amino acid sequences of TNFR1 and TNFR2 are similar in length, the apparent molecular weight is different, about 55–60 kDa and 70–80 kDa for TNFR1 and TNFR2, respectively. This difference is mainly caused by variations in **glycosylation**, as TNFR2 is highly *O*-glycosylated in the so-called stalk region, that is, between the fourth cysteine-rich domain and the transmembrane part. The stalk regions of both TNFR1 and TNFR2 contain proteolytic cleavage sites. Activation of metalloproteases (e.g., TACE) results in cleavage of TNFR1 and TNFR2, leading to the release of the respective extracellular domains. These are still capable of binding to TNF, although with lower affinity, as compared to the full-length transmembrane TNFR. Under many pathological conditions, the levels of soluble TNFR are elevated in patients. In general, higher levels of soluble TNFR2 (2–4 ng/ml) are found in normal sera as compared to soluble TNFR1. The physiological function of shed TNFR are not fully understood; neutralization of the ligand and/or formation of storage pools are plausible.

The main sources of TNF synthesis are monocytes and macrophages, although many other cell types are also able to produce TNF upon stimulation. Many proinflammatory activities of TNF are mediated by TNFR1, which is ubiquitously expressed. In contrast, TNFR2 is highly regulated in its expression and mainly found on immune cells, endothelial cells, and neurons. TNF strongly induces gene expression, although, dependent on the cellular environment, it is also capable of initiating apoptosis as well as necrosis. Whereas TNFR1 is fully activated by both TNF forms, that is, soluble and membrane bound TNF, TNFR2 can be efficiently activated only by membrane bound TNF. Crystal structure analysis of lymphotoxin α (LT α), another ligand of both TNFR, in complex with the extracellular domain of TNFR1 revealed that the homotrimeric ligand binds three receptors. However, further oligomerization of TNFR/ligand complexes is essential for efficient signaling. Upon appropriate activation, both TNFR initiate a complex series of signaling pathways (Fig. 2), including the activation of c-jun N-terminal kinase (JNK), mitogen activated protein (MAP) kinases, and the transcription factor nuclear factor- κ B (NF- κ B) [2]. Additionally, TNFR1



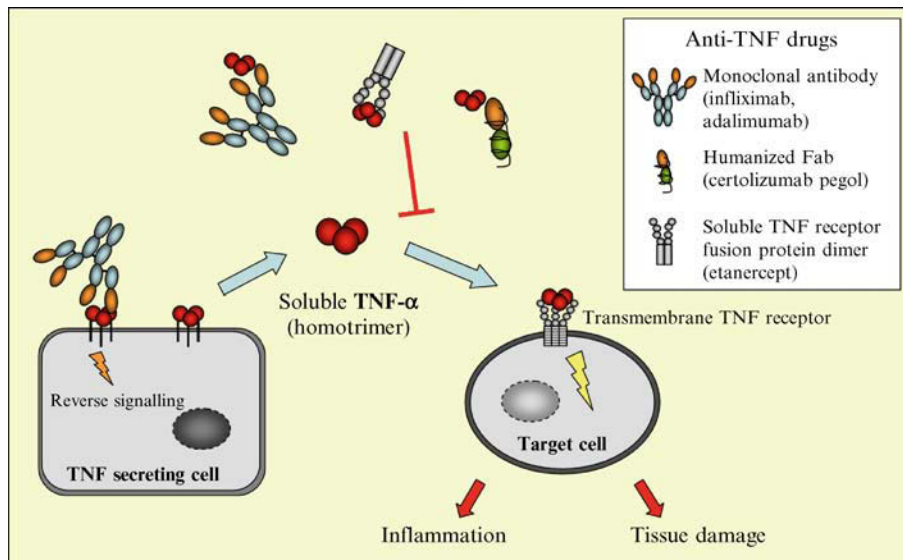
Tumor Necrosis Factor. Figure 2 Schematic representation of TNFR1- and TNFR2-mediated signaling. Activation of TNFR1 and TNFR2 by TNF regulates the expression of a number of genes affecting cellular responses to TNF. Although TNFR2 is not capable to induce the apoptotic pathway directly, the internalization of the TNFR1 signaling complex can lead to the recruitment and activation of caspases, resulting in the induction of apoptosis. Alternatively, TNFR1 can induce cell death by the induction of necrosis (not depicted here). A large number of different signaling molecules as well as kinases are involved in TNFR-mediated signaling. FADD, Fas-associated death domain; JNK, c-jun N-terminal kinase; IKK, I κ B kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; MAPKK/MKK, mitogen-activated protein kinase kinase; NF- κ B, nuclear factor kappa B; RIP, receptor-interacting protein; TAK1, TGF- β -activated kinase 1; TRADD, TNFR1-associated death domain; TRAF2, TNFR-associated factor.

activates protein kinase C (PKC) as well as sphingomyelinases that lead to the production of the lipid second messenger ceramide. After ligand binding, two different and sequential signaling pathways are activated. First, the membrane-expressed signal complex induces activation of MAPK, JNK, and NF- κ B. After internalization of the complex, a second signal pathway is initiated, leading to the induction of apoptosis. Activation of the latter is initiated by the proteolytic activation of the ▶caspase cascade. Importantly, TNFR2 is a mere gene-inductive receptor that is able to antagonize TNFR1-mediated apoptotic signaling by upregulation of the expression of antiapoptotic factors. However, in some cellular systems TNFR2-mediated signaling can lead to the expression and production of TNF that in turn is capable to activate TNFR1, thereby enhancing the proapoptotic effect of this receptor. Taken together, TNF signaling via the two TNFR, TNFR1 and TNFR2, and the crosstalk between these receptors are complex and cell-type dependent [2].

Drugs

TNF is a pleiotropic ▶cytokine exerting a wide range of cellular responses, that affect biological processes such as lipid metabolism, coagulation, and insulin resistance and the function of endothelial cells. As a major proinflammatory ▶cytokine TNF is also involved in progression of diseases like cancer, Alzheimer, Diabetes type II, cardiovascular, pulmonary or neurological disorders, and many autoimmune diseases. Blocking the action of TNF clearly reduces its inflammatory potential on various autoimmune disorders like Crohn's disease, rheumatoid arthritis (RA), and psoriasis.

Commercially available drugs used for therapeutic therapy comprise up to date mainly injectable monoclonal antibodies like Infliximab (Remicade®) and Adalimumab (Humira®) or TNF-receptor derivatives like Etanercept (Enbrel®) (Fig. 3). One possible way of action of these reagents is the neutralization of TNF, thereby blocking its inflammatory effects and dampening (auto)immune responses [3, 4].



Tumor Necrosis Factor. Figure 3 Anti-TNF drugs. Secreted soluble TNF binds to target cells via transmembrane receptors. Upon binding of TNF to TNFR1, TNF is able to induce inflammation, which may enhance pathological symptoms and tissue damage. TNF-specific monoclonal antibodies, PEGylated anti-TNF Fab fragments or TNF receptor fusion proteins are able to bind TNF, thereby blocking the accessibility of TNF to TNF receptors on target cells, resulting in the neutralization of the proinflammatory potential of TNF (modified from Ref [4]).

Neutralizing Anti-TNF Drugs

Infliximab is mainly used in treatment of psoriasis and arthritis of psoriasis. Infliximab is a chimeric monoclonal antibody consisting of 75% human IgG1 Fc and 25% mouse IgG1 containing the TNF-binding domain. Infliximab binds and neutralizes both secreted TNF and transmembrane TNF, the latter inducing reverse signaling into the TNF-secreting cells. Additionally, there are approved concepts applying the combination of Infliximab with antimetabolite drugs, such as ►methotrexate (Rheumatrex, Trexall) [4]. The precise mechanism of action of this combined therapy in diseases is currently unknown and has to be elucidated. As a result of its potentiating effects, ►methotrexate has been found helpful in treating certain diseases associated with abnormally rapid cell growth, such as cancer of the breast and psoriasis. Furthermore, infliximab alone has been reported to be helpful in decreasing the severity of symptoms of Crohn's disease, the joint ►inflammation of juvenile RA, ankylosing spondylitis, uveitis, and sarcoidosis, all known to be rather unresponsive to traditional therapies. However, it has to be kept in mind that infliximab is mainly easing and not curing these diseases.

Adalimumab (Humira®) is a fully human anti-TNF monoclonal antibody that is mainly used in combination with ►methotrexate to treat rheumatoid arthritis in adults [3]. Meanwhile, it is also used against arthritis of psoriasis and ankylosing spondylitis. Its therapeutic value in Crohn's disease is under clinical trial.

Etanercept (Enbrel®) is a chimeric TNF receptor 2 (TNFR2/p75/CD120b) IgG fusion protein consisting of two extra cellular domains of TNFR2 for ligand binding fused to the Fc-part of human IgG1. It is used to reduce the signs and symptoms of moderately to severely active juvenile RA in patients, who had shown an inadequate response to other disease-modifying medications [3]. Etanercept is further approved for the treatment of ankylosing spondylitis and the arthritis of psoriasis. It can prevent the progressive destruction of the joints in patients with psoriatic arthritis. Therefore, Etanercept can improve physical function in patients that are restrained by psoriatic arthritis. It has also been reported to benefit in psoriasis and uveitis. However, there are restrictions that Etanercept is not recommended for patients with preexisting disease of the CNS (central nervous system) or for those with MS (multiple sclerosis), myelitis, or optic neuritis.

In addition to therapeutics in use already, there is a fourth upcoming anti-TNF drug, called the TNF-neutralizing drug Certolizumab pegol (CDP870). It is a ►PEGylated Fab fragment of a humanized anti-TNF monoclonal antibody (Fig. 3). At present, it is being tested in clinical phase III trials for both Crohn's disease and RA. Previous studies suggest that it is well tolerated by patients.

In addition to the alleviation of symptoms, the therapeutic use of these drugs results in some side effects. Often, depending on the setup of treatment, flu-like symptoms such as shivering, fever, aching muscles,

weakness, loss of appetite, nausea, vomiting, and diarrhea are observed. Some patients show a breakout of a rash around the area of injection, others tend to bleed or bruise easily. Depending on how severe these problems are, patients may need to stay in hospital during the period of treatment. These side effects usually occur short-term and they gradually reduce after treatment stops.

Furthermore, it also has to be considered that TNF-neutralizing drugs may weaken the innate immune system. In fact, there exists the risk of contracting other diseases, such as tuberculosis. Especially, latent infections may become active, often leading to an atypical clinical presentation of the disease. Infliximab and Adalimumab have label warnings which state that patients should be evaluated for latent tuberculosis infection and treatment should be initiated prior to starting therapy with these medications. Additionally, anti-arthritis drugs like TNF blockers might also allow cancer to occur as they suppress the immune system's ability to fight off tumors.

The Antitumoral Activity of TNF

TNF was originally identified because of its cytotoxic activity against some tumor cell lines and its ability to induce hemorrhagic necrosis of solid tumors in various animal models. However, the clinical use of TNF as an anticancer drug has been so far limited by its severe cardiovascular side effects. Therefore, TNF treatment is limited to regional and local administration of high doses of TNF, often in combination with chemotherapy, as accomplished in isolated limb and isolated hepatic perfusion (ILP and IHP, respectively) [5]. In the case of ILP, typically metastases are treated, patients benefit from this procedure by salvage of limbs from a loss by amputation.

TNF Prodrugs as Antitumoral Medication

A different upcoming approach is the use of TNF ►prodrugs. Thereby, the properties of TNF and TNF receptors are used to achieve a direct targeting via a fusion protein construct. An agonistic peptide consisting of 11 amino acids, comprising the TNF binding site of TNFR1, has been shown to possess many of the leukocyte-activating properties of TNF without the associated toxicity when administered locally or systemically in an animal model.

In a different approach, a TNF fusion protein designated TNF-Selectokine has been described [5]. This TNF ►prodrug is comprised of a trimeric scFv-TNF fusion protein to which a TNFR fragment has been fused at the C-terminal. A flexible peptide linker between TNF and the blocking receptor domain is comprised of sequences specifically recognized by tumor associated proteases such as tissue plasminogen activator, urokinase type plasminogen activator or

matrix metalloproteinase-2 (MMP2). The TNF-Selectokine exerts high bioactivity toward the targeted and adjacent antigen negative cells [5]. Unfortunately, available data are so far from animal studies, clinical trials have to be performed to verify these effects in humans.

- Tumor Necrosis Factor α
- TNF Receptor Associated Factors
- TNF Receptor Superfamily

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Tumor Suppressor Genes

In multicellular organisms, the proteins encoded by tumor suppressor genes exert protective effects against malignant transformation. Their main functions are (i) inhibition of cell cycle leading to reduced proliferation, (ii) promotion of apoptosis, (iii) coordination of the cellular response to DNA damage. A large number of tumors show loss or inactivation of both alleles of a given tumor suppressor gene.

TUNEL

TUNEL is the name given to the in-situ DNA end-labelling technique which serves as a marker of apoptotic cells. This method is based on the specific binding of terminal de-oxy nucleotidyl transferases to the 3-hydroxy ends of DNA. The technique is normally used for examination under the light microscope but can also be adapted for examination under the electron microscope.

- Apoptosis

Turing Machine

The Turing machine is one of the key abstractions used in modern computability theory. It is a mathematical model of a device that changes its internal state and reads from, writes on, and moves a potentially infinite tape, all in accordance with its present state. The model of the Turing machine played an important role in the conception of the modern digital computer.

► Bioinformatics

Twitch/Tetanus

Twitch is a muscle contraction caused by a single action potential, whereas tetanus is a sustained muscle contraction caused by a series of repetitive action potentials. The amplitude of tetanus contraction is larger than that of twitch, due to mechanical summation.

► Ryanodine Receptor

Two-dimensional Gel Electrophoresis

Two-dimensional gel electrophoresis (2DE) is a two-dimensional technique for protein separation, which combines isoelectric focusing and sodium dodecyl sulphate (SDS) electrophoresis. The high resolving power results from separation according to charge (isoelectric point) in the first dimension and size (mobility in a porous gel) in the second dimension. Depending on the gel size, from several hundred to more than 5,000 proteins can be separated.

► Proteomics

Two Metal Sites

Two metal sites were identified in the structure solved of the chimeric **AC5C₁·AC2C₂** adenylyl cyclase in complex with β -L-2',3'-dd-5'-ATP (cf. Figs. 5 and 7) One is

associated with the pyrophosphate moiety of the inhibitor and the other coordinates the attack of the 3'-OH group at the α -phosphate, thereby catalyzing the cyclizing reaction, when 5'-ATP is bound. Figure 7 depicts structures of nucleotides and divalent cation as extracted from their respective crystal structures with **AC5C₁·AC2C₂**.

► Adenylyl Cyclases

L-Type Ca²⁺ Channel

The dihydropyridine-sensitive Ca_v1.2 calcium channel, that is essential for smooth muscle contraction and the target for the calcium channel blocker/calcium antagonists.

► Smooth Muscle Tone Regulation
 ► Voltage-dependent Ca²⁺ Channels
 ► Ca²⁺ Channel Blockers

Type I Allergic Reaction

Type I allergic reactions are inappropriate immune responses to an allergen with preferential synthesis of immunoglobulin E (IgE), a special antibody class, which binds to mast cells and basophilic granulocytes via Fc ϵ receptors. Binding of the allergen to the cell-bound IgE initiates the rapid release of allergic mediators, most prominently histamine, and the de novo synthesis of arachidonic acid metabolites and cytokines, which are responsible for the clinical symptoms.

► Allergy

Type I Transmembrane Proteins

Cell membrane spanning proteins contain a luminal/extracellular domain, a transmembrane region and a cytosolic domain. In a type I transmembrane protein the N-terminus is the extracellular/luminal part of the protein, whereas the C-terminus comprises the cytosolic region of the membrane protein.

► Tumor Necrosis Factor (TNF)

Type II Allergic Reaction

A Type II allergic reaction occurs when antibodies specific for foreign substances recognize the body's own cells after they have firmly bound these foreign substances and initiate the cell's destruction by immune mechanisms.

► Allergy

Type II Transmembrane Proteins

Cell membrane spanning proteins contain a luminal/extracellular domain, a transmembrane region and a cytosolic domain. In a type II transmembrane protein the C-terminus is the extracellular/luminal part of the protein, whereas the N-terminus comprises the cytosolic region of the membrane protein.

► Tumor Necrosis Factor (TNF)

Type III Allergic Reaction

A Type III allergic reaction occurs when antibodies of the immunoglobulin G class (IgG) form immune complexes which are slowly eliminated and thus may elicit an inflammatory reaction by binding to the Fcγ receptors of leukocytes resulting in their activation.

► Allergy

Type IV Allergic Reaction

Type IV allergic reactions are cell-mediated hypersensitivity reactions which are characterized by the expansion of T lymphocytes specific for foreign substances exposed on cell surfaces. In type IVa allergic reactions, this results in the cell-mediated destruction of the cells, whereas in type IVb allergic reactions an

inflammatory reaction results after release of cytokines (delayed-type hypersensitivity reaction, DTH).

► Allergy

Typical Antipsychotic Drugs: Neuroleptic Drugs

► Antipsychotic Drugs

Tyrosine Hydroxylase

Tyrosine hydroxylase (TH) is an enzyme that catalyzes the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine in the brain and adrenal glands. TH is the rate-limiting enzyme in the biosynthesis of dopamine. This non-heme iron-dependent monooxygenase requires the presence of the cofactor tetrahydrobiopterin to maintain the metal in its ferrous state.

► Dopamine System

Tyrosine Kinase Inhibitors

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Synonyms

TKIs (tyrosine kinase inhibitors); Kinase inhibitors; Monoclonal antibodies

Definition

Tyrosine kinase inhibitors interfere with the function of tyrosine kinases that catalyze the transfer of the γ-phosphate group of ATP to tyrosine residues of protein substrates. Tyrosine kinases can be subdivided into two large families, receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs; see corresponding chapter). The human genome

encodes for a total of 90 tyrosine kinases that have been implicated in the regulation of a variety of biological responses such as cell proliferation, migration, differentiation and survival. There is evidence that they are involved in the development of many diseases, including immunodeficiency, inflammation, ►atherosclerosis, psoriasis, ►osteoporosis, diabetes and cancer.

Mechanism of Action

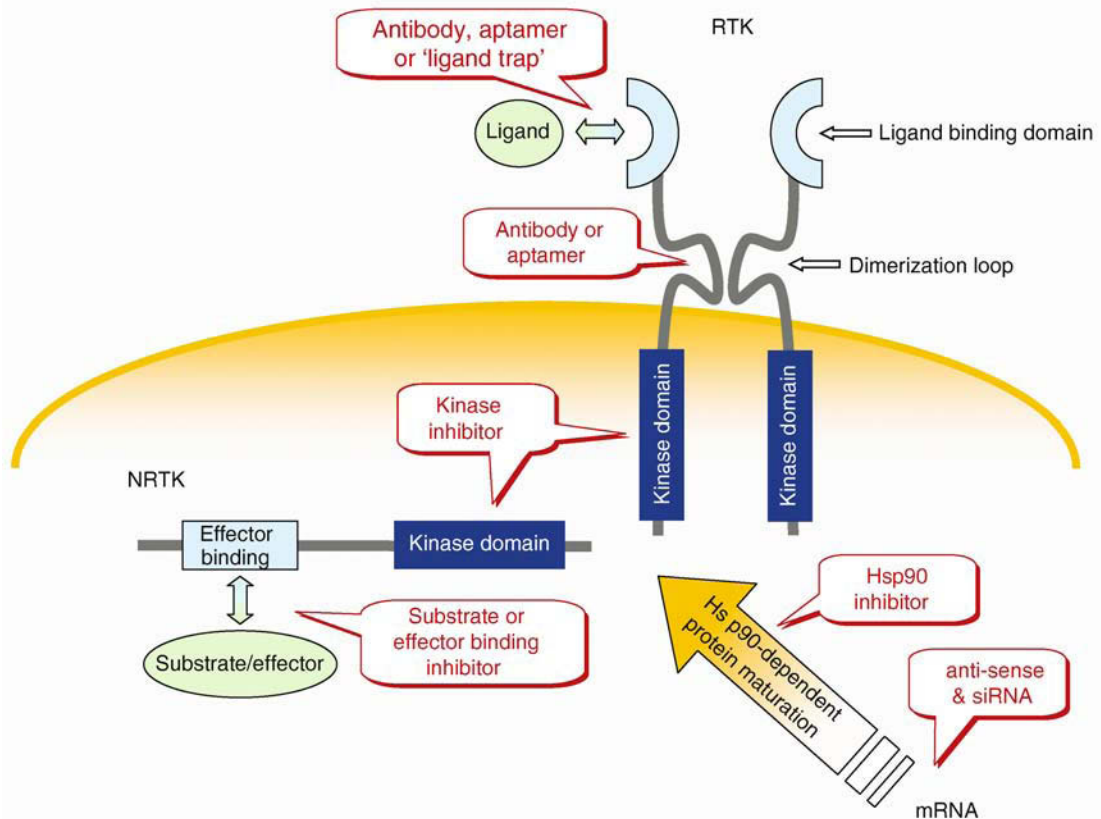
Tyrosine kinases can be targeted by different pharmacological strategies. Both, RTKs and NRTKs are accessible to small molecule inhibitors directly interfering with ATP binding and to Hsp90 ►chaperone inhibitors that affect maturation of several kinases following their synthesis. Furthermore, they can be equally addressed by mRNA directed approaches, such as anti-sense therapy (►antisense oligonucleotide) and ►RNA interference (RNAi). In addition, RTKs are targeted by monoclonal antibodies, “ligand traps” and ►aptamers that block binding of activating ligands, interfere with dimerization that is part of the activation process, or induce down-regulation of the receptor protein (Fig. 1). Furthermore, some anti-RTK antibodies may also trigger ►antibody-dependent cellular

cytotoxicity (ADCC) that can contribute to their therapeutic activity.

Antibodies

The first drug targeting a tyrosine kinase approved for clinical use was the anti-ErbB2/Her-2 antibody trastuzumab (Herceptin) that is successfully applied for the treatment of Her-2 positive breast cancers. Her-2 expression is assessed in tumor biopsy material prior to treatment either by immunohistochemistry or ►fluorescence in situ hybridization (FISH). Current data support the hypothesis that trastuzumab exerts its therapeutic effects by inhibition of ErbB2/Her-2 shedding and induction of receptor down-regulation. A second anti-Her-2 antibody that is currently tested in clinical studies is pertuzumab (Omintarg). This antibody is directed against the dimerization domain of Her-2 and inhibits homo- and heterodimerization.

A variety of antibodies has been developed against the epidermal growth factor receptor (EGFR), another ErbB family member that is overexpressed in a several human cancers, but has also been implicated in other diseases like psoriasis. The chimeric antibody cetuximab interacts with domain III of EGFR, prevents binding of activating ligands and can as an IgG₁ elicit



Tyrosine Kinase Inhibitors. Figure 1 Pharmacological strategies to target tyrosine kinases.

ADCC. Cetuximab is approved for treatment of metastatic colorectal cancer (CRC) and squamous cell carcinoma of the head and neck (SCCHN). Interestingly, an adverse event, acneiform rash seems to correlate with a better response to cetuximab, while there is no such correlation with expression levels of EGFR assessed by immunohistochemistry. Further side effects are rare infusion reactions and hypomagnesia. Two other anti-EGFR antibodies approved for clinical use are the fully human antibody panitumumab (Vectibix)

that has been generated in mice transgenic for human IgGs and the humanized antibody nimotuzumab (TheraCIM) (Table 1). Remarkably, nimotuzumab seems to be the only EGFR-targeting drug without reports about skin rash. Additional anti-EGFR antibodies in clinical trials are the fully human IgG zalutumumab (HuMax-EGFR), the humanized matuzumab (EMD72000) and the chimeric antibody ch806. While all other antibodies bind with high affinity to wildtype EGFR, ch806 seems to recognize only a

Tyrosine Kinase Inhibitors. Table 1 Tyrosine kinase inhibitors approved for clinical use (by July 2007)

Drug name	Drug format	Main target(s)	MoA	Indication*
Trastuzumab (Herceptin)	Humanized antibody, IgG ₁	ErbB2/Her-2	Receptor down-regulation	Breast cancer
Cetuximab (Erbix)	Chimeric antibody, IgG ₁	EGFR	Ligand competition ADCC ?	CRC SSCHN
Panitumumab (Vectibix)	Fully human antibody, IgG ₂	EGFR	Ligand competition	CRC
Nimotuzumab (TheraCIM)	Humanized antibody, IgG ₁	EGFR	Ligand competition ?	SSCHN
Gefitinib (Iressa)	TKI	EGFR	Inhibition of kinase activity – ATP-competitive	NSCLC
Erlotinib (Tarceva)	TKI	EGFR	Inhibition of kinase activity – ATP-competitive	NSCLC Pancreatic cancer
Lapatinib (Tykerb)	TKI	EGFR, ErbB2/Her-2, ErbB4	Inhibition of kinase activity – ATP-competitive	Breast cancer
Bevacizumab (Avastin)	Humanized antibody, IgG ₁	VEGF	Inhibition of ligand binding	CRC NSCLC
Ranibizumab (Lucentis)	Humanized antibody fragment, IgG ₁	VEGF-A	Inhibition of ligand binding	AMD
Pegaptanib (Macugen)	Aptamer	VEGF-165	Inhibition of ligand binding	AMD
Sorafenib (Nexavar)	TKI	Raf, VEGFR2, c-Kit, PDGFR, Flt-3, FGFR1	Inhibition of kinase activity – ATP-competitive	RCC
Sutinib (Sutent)	TKI	VEGFR2, c-Kit, PDGFR, Flt-3, FGFR1	Inhibition of kinase activity – ATP-competitive	RCC GIST
Imatinib (Gleevec)	TKI	Bcr-Abl, c-Kit, PDGFR, Lck	Inhibition of kinase activity – ATP-competitive, binding to an inactive conformation	CML ALL GIST DFSP MDS/MPD ASM HES/CEL
Dasatinib (Sprycel)	TKI	Bcr-Abl, Src family kinases, c-Kit, PDGFR, EphB4	Inhibition of kinase activity – ATP-competitive, binding to an active conformation	CML ALL

*Note that application in the particular indications is usually restricted either to patients expressing the target (e.g. trastuzumab, cetuximab, lapatinib, imatinib) and/or after failure of prior therapies (e.g. cetuximab, erlotinib, lapatinib, sutinib, dasatinib). Furthermore, for cancer treatment most tyrosine kinase inhibitors are applied in combination with conventional chemotherapeutic drugs, such as fluorouracil, taxanes, platin-based regimens, anthracyclines and irinotecan or radiotherapy.

fraction of EGFR on cells highly overexpressing the receptor and a particular EGFR mutant called variant III that is frequently found in brain tumors. Whether this property translates to an improved safety profile by retaining similar efficacy than approved anti-EGFR antibodies remains to be demonstrated in clinical trials.

Another antibody-based approach targets vascular endothelial growth factor (VEGF) that has been implicated in tumor angiogenesis and other pathological neovascularization processes. In contrast to anti-EGFR strategies, RTK activation is in this case prevented by interference with the ligand and not with the RTK. Bevacizumab (Avastin) has changed the standard of care for patients with metastatic CRC by significantly increasing their survival. It has also been approved for non-small cell lung cancer (NSCLC) and due the fundamental mechanism of action an impact on other cancer treatments can be expected. Bevacizumab is generally well tolerated, though, some serious and unusual toxicities were reported (e.g. gastrointestinal perforations, wound healing complications, hypertension, bleeding and proteinuria). Ranibizumab (Lucentis) is a fragment of bevacizumab engineered specifically to penetrate the retina and is approved for the treatment of ►age-related macular degeneration (AMD) where it stabilizes vision in more than 90% of wet AMD patients.

Given the success of this class of therapeutics a number of antibodies directed against RTKs (e.g. fibroblast growth factor receptors (FGFRs), insulin-like growth factor 1 receptor (IGF1R) and VEGFR) and RTK ligands (e.g. hepatocyte growth factor) are being developed.

Kinase Inhibitors

Though a large number of small molecule inhibitors against a variety of tyrosine kinases are being developed, the most advanced drugs can be roughly classified as (i) Bcr-Abl/Src family kinase inhibitors, (ii) ErbB inhibitors and (iii) broad spectrum TKIs with an anti-angiogenic component.

Among the Bcr-Abl inhibitors is *the* forerunner for the TKI approach, imatinib (STI-571, Gleevec) that shows remarkable clinical activity in patients with chronic myelogenous leukemia (CML) and in a subset of acute lymphoblastic leukemia (ALL). In both diseases Bcr-Abl, a fusion protein of the NRTK c-Abl and the breakpoint cluster region (bcr) with elevated kinase activity is the causative molecular abnormality. Imatinib is in general well tolerated, though, more recently a cardiotoxic potential has been described. As with almost all anti-cancer drugs, primary and acquired resistance to imatinib is observed. Most important mechanisms for resistance are Bcr-Abl amplifications and mutations in the kinase domain that reduce affinity for the drug. Imatinib resistance in CML and ALL is addressed by second generation c-Abl inhibitors

like nilotinib (AMN-107, Tasisign) and dasatinib (BMS-354825, Sprycel). Imatinib is also used for the treatment of gastrointestinal stroma tumors (GIST), dermatofibrosarcoma protuberans (DFSP), myelodysplastic/myeloproliferative diseases (MDS/MPD), aggressive systemic mastocytosis (ASM) and hyper-eosinophilic syndrome/chronic eosinophilic leukemia (HES/CEL). The clinical activity of Imatinib in GIST is based on its ability to inhibit c-Kit, which is frequently mutated in this disease. Other indications, such as DFSP, HES/CEL and a certain proportion of CML involve aberrant PDGF receptor signaling that is blocked by imatinib.

Several TKIs against the ErbB family members EGFR and ErbB2/Her-2 have been generated. While gefitinib (ZD1839, Iressa) and erlotinib (OSI-774, CP-358774, Tarceva) are relatively selective for EGFR, lapatinib (GW572016, Tykerb) was designed as a dual EGFR and ErbB2/Her-2 inhibitor. Gefitinib and erlotinib are used for the treatment of NSCLC and recent data suggest that patients with certain somatic EGFR kinase domain mutations have a particular benefit from this regimen. Lapatinib is approved for the treatment of patients with Her-2 overexpressing metastatic breast cancer who have received prior therapies including trastuzumab.

Examples for the ever-growing class of multi-kinase inhibitors with anti-angiogenic activity are sunitinib (SU11248, Sutent), sorafenib (BAY 43-9006, Nexavar), vandetanib (ZD6474, Zactima), cediranib (AZD2171, Receptin), vatalanib (PTK787), pazopanib (GW786034) and axitinib (AG013736). A common feature of all these TKIs is their activity against one or several RTKs involved in angiogenesis, such as VEGFR1, VEGFR2 and PDGFR. This property may translate to a wide spectrum of cancer indications and first remarkable results led to the approval of sunitinib and sorafenib in renal cell cancer (RCC). This clinical activity may be explained by the frequent loss of the Van Hippel Lindau (VHL) gene in RCC that results in the accumulation of hypoxia-induced factor (HIF) and subsequent activation of HIF-inducible pro-angiogenic proteins, such as VEGF and PDGF. Interestingly, sorafenib was originally developed as a Raf serine/threonine kinase inhibitor, but initially failed in clinical studies in melanoma that frequently express a mutant, activated variant of B-Raf. In addition, many of these TKIs share activity against c-Kit predestining them for the treatment of GIST with activating c-Kit mutations. Again, these TKIs are relatively well tolerated despite their broad target profile.

More TKIs directed against other tyrosine kinases, like c-Met, IGF1R, Flt-3, FGFRs, Tie-2, Axl or Ron are at earlier stages of development with the most advanced candidates having entered phase II studies in different types of cancers.

Hsp90 Inhibitors

Hsp90 is a heat-shock protein that functions as molecular chaperone guiding normal protein folding, intracellular deposition and proteolytic turnover of many key regulators of cell growth and survival. Among the client proteins of Hsp90 are several tyrosine kinases, such as Her-2, c-Kit, Flt-3, Bcr-Abl and mutated Src that are involved in oncogenesis. Therefore, Hsp90 inhibitors are clinically tested for the treatment of cancers, but preclinical data also suggest a potential use in arthritis and neurodegenerative disorders, like Parkinson and Alzheimer disease. The most advanced compounds, 17-AAG (Tanespimycin) and 17-DMAG are derived from natural products and target the N-terminal ATP-binding pocket of Hsp90. While there were initial concerns about severe side effects due to impairment of normal cell function, clinical phase I studies point to an acceptable safety profile of these drugs. Recognized as an attractive target, several fully synthetic small molecule Hsp90 inhibitors suitable for oral application are approaching clinical phase I.

Others

Among the alternative approaches to target tyrosine kinases only pegaptanib (Macugen), an RNA aptamer that binds to VEGF-165, the VEGF isoform primarily responsible for pathological ocular neovascularization and vascular permeability has gained approval for the treatment of AMD.

Another strategy to interfere with the biological actions of tyrosine kinase activating growth factors is based on so called decoy receptors. These are soluble receptor proteins that contain a native ligand binding domain able to capture the growth factor and thereby prevent activation of its cognate transmembrane receptor(s). An example for such as “ligand trap” is the VEGF trap (Aflibercept), a genetically engineered fusion protein of the extracellular domain of VEGFR1 and the constant region of human IgG₁. This construct binds all forms of VEGF-A with high affinity and shows promising clinical activity in the treatment of several cancers as well as in AMD.

Furthermore, peptidomimetic SH2 domain inhibitors for Src, such as AP-22408 have been designed that interfere with effector binding and thereby disrupt signal transduction. AP-22408 decreases bone resorption in animal studies and may be a promising drug to treat osteoporosis and other bone diseases, such as Paget's disease and osteolytic bone metastasis.

Other approaches directed to the mRNA of tyrosine kinase and thereby interfering with their translation to proteins, such as anti-sense therapy and RNA interference (RNAi) are less advanced and currently limited to laboratory studies.

Clinical Use

Tyrosine kinase inhibitors that are currently in clinical use either target ErbB family members, VEGF and its receptors or Bcr-Abl (Table 1). They are applied for the treatment of different types of cancers and for AMD. While antibody-based therapeutics and aptamers are highly selective for their primary target, most small molecular kinase inhibitors have a broader profile ranging from a few additional kinases that are affected (e.g. imatinib and gefitinib) to a significant promiscuity (e.g. sunitinib and sorafenib). Main reason for limited selectivity is that most TKIs are directed against the ATP binding pocket that is to a certain degree conserved throughout the kinome. While this initially raised concerns about potential side effects, hitting several kinases with a single drug can allow the exploration of additional indications (e.g. imatinib for GIST due to its activity against c-Kit) or may even provide a general advantage for a multi-causal disease like cancer. Clinical studies in other indication will show whether the lack of selectivity of many TKIs is an obstacle for the treatment of less life-threatening diseases than cancer.

► Growth Factors

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Tyrosine Kinases

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Synonyms

Protein tyrosine kinases; Nonreceptor tyrosine kinases; Cytoplasmic tyrosine kinases; Tyrosylprotein kinase; Hydroxyaryl-protein kinase

Definition

Protein tyrosine kinases (PTKs) are enzymes (EC 2.7.1.112) that catalyze the transfer of the γ -phosphate group of ATP to tyrosine residues of protein substrates. The activity of PTKs is controlled in a complex manner by posttranslational modifications and by inter- and intramolecular complex formations.

Basic Characteristics

PTKs have been implicated in the regulation of a variety of biological responses such as cell proliferation, migration, differentiation, and survival. They have been demonstrated to play significant roles in the development of many disease states, including immunodeficiency, **atherosclerosis**, **psoriasis**, **osteoporosis**, diabetes, and cancer. In recent clinical trials impressive antitumor effects of PTK inhibitors have been observed. In future, PTK inhibitors may therefore become important drugs for the treatment of specific cancers.

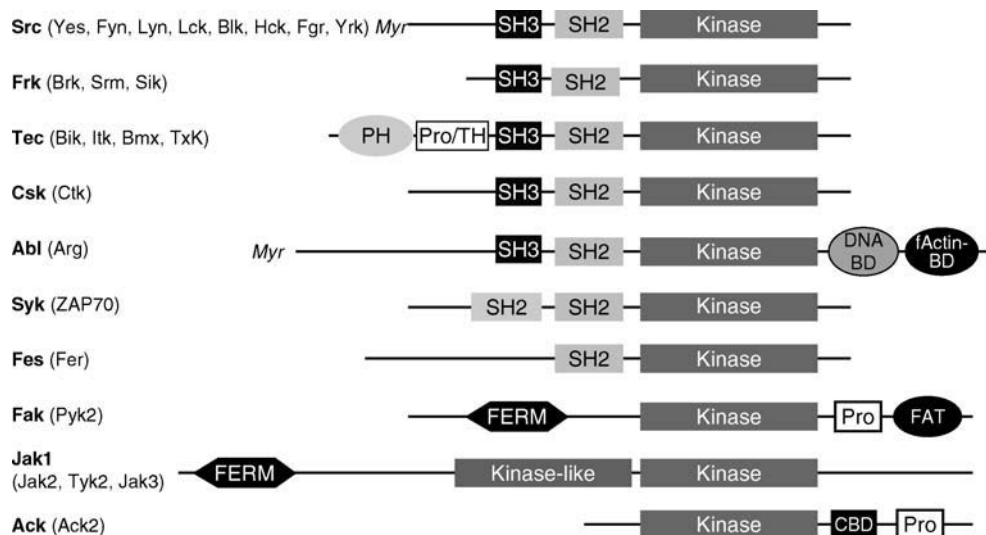
PTKs can be subdivided into two large families, receptor tyrosine kinases (RTKs) and non-RTKs. The human genome encodes for a total of 90 tyrosine kinases of which 32 are nonreceptor PTKs that can be placed in 10 subfamilies (Fig. 1). All nonreceptor PTKs share a common kinase domain and usually contain several additional domains that mediate interactions with protein-binding partners, membrane lipids, or DNA (Table 1). These interactions may affect cellular localization and the activation status of the kinase or attract substrate proteins for phosphorylation reactions.

c-Src

c-Src was the first cellular homologue of a viral oncoprotein (v-Src from the Rous sarcoma virus) to

be discovered. It is involved in mitogenic signaling from many types of transmembrane receptors and has been implicated in a variety of cancers. c-Src and the Src-like kinases Fyn and Yes are expressed in most tissues and are at least partially redundant in their function. Hck, Fgr, and Blk are primarily found in hematopoietic cells, whereas Lyn and Lck are also expressed in neuronal cells.

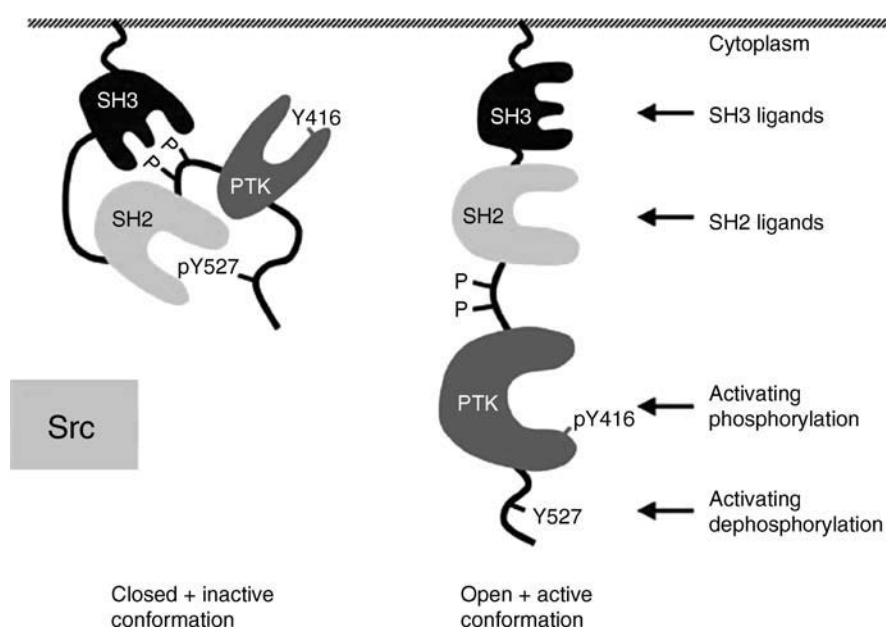
The inactive, closed conformation of Src is maintained by intramolecular interactions of the SH2 and SH3 domains (Fig. 2). The N-terminal SH3 domain binds to a proline-rich sequence in the linker region between the SH2 and the kinase domain. In addition, the SH2 domain binds to a phosphorylated tyrosine residue (Y527 in chicken, Y530 in human) in the C-terminal part of the protein. The kinase executing this phosphorylation is called Csk (C-terminal c-Src kinase) and is member of a distinct PTK family (Fig. 1). Both intracellular interactions together repress Src kinase activity by blocking access to the active site. Src can be activated by dephosphorylation of pY527 and by intermolecular interactions with SH2 and SH3 binding partners. A variety of cytosolic and receptor-type protein tyrosine phosphatases (PTPs), such as PTP α , PTP1B, and SHP-1/2 has been shown to dephosphorylate pY527 and subsequently activate c-Src. Among the SH2 ligands that can activate Src are autophosphorylated RTKs (e.g., epidermal growth factor (EGF) and platelet derived growth factor (PDGF) receptors) and nonreceptor PTKs (e.g., Fak and Pyk2) as well as tyrosine phosphorylated adaptor proteins (e.g. Shc). For full activation of Src a transautophosphorylation of a conserved tyrosine residue in the activation loop (Y416) has to occur. This model is supported by the elevated Src activity in transformed cells with



Tyrosine Kinases. Figure 1 Tyrosine kinases.

Tyrosine kinases. Table 1 Tyrosine kinases

Domain		Function
CBD	Cdc42-binding domain	Binding to the small G protein Cdc42
DNA-BD	DNA-binding domain	Binding to DNA
fActin-BD	f-Actin-binding domain	Binding to F-Actin
FAT	Focal adhesion targeting domain	Binding to focal adhesions complexes
FERM	4.1/ezrin/radixin/moesin domain	Binding to cytoplasmic regions of transmembrane proteins
Myr	Myristoylation site	Tethering to membranes
PH	Pleckstrin homology domain	Binding to membrane phospholipids, such as phosphoinositides
Pro	Prolin-rich sequences	Binding to SH 3 domains
PTB	Phospho-tyrosine binding domain	Binding to phosphorylated tyrosine residues
SH1	Src homology 1 domain, Kinase domain	Kinase activity
SH2	Src homology 2 domain	Binding to phosphorylated tyrosine residues
SH3	Src homology 3 domain	Binding to prolin-rich sequences
TH	Tec homology domain	SH3-binding prolin-rich sequences and Zn ²⁺ -binding motif

**Tyrosine kinases. Figure 2** Activation mechanism of Src.

increased PTP activity; a Src mutant with truncated C-terminus lacking Y530 that has been found in human colorectal cancers and is constitutively activated, and v-Src, which in addition to several point mutations lacks a large part of the C-terminal domain, has transforming potential.

Among the substrates of Src are other nonreceptor PTKs (e.g., Fak, Syk, and Tec kinases), RTKs (e.g. EGF and PDGF receptors), phospholipase C γ , PI3-kinase, phosphatases (e.g., SHP-2 and PP2A), and adaptor (e.g., Shc and Cbl) as well as focal adhesion proteins (e.g., paxillin, p130^{Cas} and tensin). Src-mediated phosphorylation either modulates enzymatic activity of

target proteins or creates docking sites for SH2 or PTB domain containing proteins promoting the assembly of multimeric protein complexes that function in cellular signaling.

Given its involvement in many receptor-mediated signaling pathways, Src was thought to be an important regulator of cell proliferation, migration and adhesion. However, the most striking phenotype of c-Src deficient mice is an osteopetrosis suggesting a role for c-Src in bone remodeling and a compensation of c-Src-deficiency by other Src family members in other organs. Indeed, combined deletion of c-Src, Yes and Fyn in mice results in a lethal phenotype.

c-Abl

c-Abl was first identified as the cellular homologue of the transforming gene product of the Abelson murine leukemia virus (v-Abl) and found to encode a nonreceptor PTK. Mammalian c-Abl is expressed ubiquitously and in most cells is primarily localized in the nucleus where it has a role in DNA damage-induced apoptosis.

c-Abl is activated by ionizing radiation in a manner dependent on phosphorylation by ATM (ataxia telangiectasia-mutated), a nuclear protein serine/threonine kinase. c-Abl kinase activity in G0 and G1 phase of the cell cycle is repressed by binding of Rb (retinoblastoma protein) to the activation loop, which is released during S-phase when Rb becomes hyperphosphorylated by cyclin-dependent kinases. Furthermore, c-Abl is negatively controlled by intramolecular SH3 domain interactions and by SH3-binding proteins, such as Pag/MSP23 (human proliferation-associated gene/macrophage 23-kD stress protein).

Nuclear substrates of c-Abl include DNA-PK (DNA-dependent protein kinase, an enzyme critical for DNA repair), Rad51 (a homologue of bacterial RecA involved in recombination/repair by catalyzing strand exchange between homologous DNAs), the tyrosine phosphatase SHPTP1 and the p85 subunit of PI3-kinase, negatively regulating their respective activity. In contrast, c-Abl activates JNK (c-Jun N-terminal kinase) and p38 mitogen-activated protein kinases (MAPKs). c-Abl also functions in the cytoplasm, where it is involved in PDGF-induced motility responses and cell adhesion. In chronic myelogenous leukemia (CML) as well as in a subset of acute lymphoblastic leukemia (ALL) Bcr-Abl, a fusion protein of c-Abl and the breakpoint cluster region (bcr), is expressed in the cytosol of leukemic cells. This fusion protein forms homo-oligomeric complexes that display elevated kinase activity and is the causative molecular abnormality in CML and certain ALL. The transforming effect of Bcr-Abl is mediated by numerous downstream signaling pathways, including ►protein kinase C (PKC), Ras-Raf-ERK MAPK, JAK-STAT (see below), and PI3-kinase pathways.

Fak

Fak (focal adhesion kinase) is expressed in most tissues and is evolutionary conserved across species. It is activated by integrin clustering and by stimulation of several G protein-coupled receptors and RTKs. Fak is associated with focal adhesions and regulates cell spreading and migration. The kinase is essential for embryonic development since the homozygote Fak knockout is embryonic lethal. Pyk2 (proline-rich tyrosine kinase 2), the second member of the Fak kinase family has a more restricted expression pattern (primarily neuronal and hematopoietic cells) and does not localize to focal adhesions.

An early step in Fak activation is a high stoichiometry autophosphorylation of a tyrosine residue (Y397)

proximal to the kinase domain. Phosphorylated Y397 is a high affinity ligand for the SH2 domain of Src, thereby recruiting Src kinases and stimulating their catalytic activity. In a second step, several other tyrosine residues in Fak become phosphorylated, either by Fak itself or by recruited Src. As a consequence, Fak kinase activity is further increased or docking sites for SH2 domain-containing proteins are created, e.g., for Grb2-Sos complexes that link Fak to the Ras-Raf-►MAPK cascade. N-terminal sequences containing the FERM domain anchor Fak to integrins or RTKs and the C-terminal FAT domain mediates binding to cellular focal adhesions. Among the substrates of the Fak/Src complex are the adaptor proteins paxillin and p130^{Cas} and the focal adhesion-associated protein tensin that bind to C-terminal sequences of Fak and, after their phosphorylation, promote the assembly of signaling complexes at discrete sites within cells.

Fak kinases could be modulators of some aspects of human cancers and may also contribute to the development of vascular diseases involving hyperproliferation and migration of vascular smooth muscle cells.

Jak

Jak1 and 2 were identified, among others, by PCR using degenerate oligonucleotides spanning the conserved kinase domain of Src and therefore initially named Jak, for "Just another kinase." When full length clones were isolated it was recognized that they differ markedly from other PTKs by the presence of an additional (pseudo)-kinase domain of unknown function. To denote this unique feature they were renamed as "Janus kinases" in reference to the ancient two-faced Roman god. Jak1, 2, and Tyk2 are ubiquitously expressed, whereas Jak3 is predominantly found in hematopoietic cells. Jak family PTKs mediate signaling primarily downstream of cytokine receptors. In response to ligand stimulation, cytokine receptors oligomerize and recruited or constitutively bound Jak kinases become activated and phosphorylate receptors. Some of the receptors' phosphotyrosine residues subsequently bind to SH2 domains of STATs (signal transducers and activators of transcription), which are then phosphorylated by Jaks on a C-terminal tyrosine residue. This leads to STAT oligomerization through a reciprocal interaction between SH2 domains and phosphotyrosines. Dimeric STATs translocate to the nucleus where they initiate transcription of target genes. Alternatively, STATs can be activated by Src kinases that are recruited to Jak-phosphorylated cytokine receptors via their SH2 domain. Jak kinase signaling is negatively regulated by PTPs and by SOCS (suppressors of cytokine signaling) proteins that inhibit Jaks by binding to the activation loop and by targeting the kinases for protein degradation.

STAT 3 and 5 are overexpressed and/or overactivated in several human malignancies, such as breast,

non-small cell lung cancer and head and neck cancer. An aberrant activation of Jak kinases by fusion with the TEL transcription factor and subsequent constitutive dimerization has been observed in T-cell acute lymphocytic leukemia.

Tec

Tec family kinases participate in signal transduction in response to virtually all types of extracellular stimuli that are transmitted by growth factor receptors, cytokine receptors, G protein-coupled receptors, antigen-receptors, and integrins. Tec kinases are involved in the regulation of growth, differentiation, apoptosis, and cell motility. They are primarily found in hematopoietic lineages but some family members (Btk, Etk/Bmx) have a somewhat broader expression pattern. The defining feature of Tec family kinases is the presence of a PH domain at their N-terminus. The PH domain has a broad binding capacity ranging from lipid products of PI3-kinase, heterotrimeric G protein subunits ($\beta\gamma$ as well as $G\alpha_q$ and $G\alpha_{12}$), PKC isoforms (βI and δ) to STATs, and Fak kinases. These interactions may either be involved in Tec activation (phospholipids and G proteins) or recruit potential substrates of Tec kinases (PKC and STATs).

The current understanding on activation of Tec kinases fits into a two-step model. In the first step an intramolecular interaction between the SH3 domain and a proline-rich region in the TH domain is disrupted by binding of the PH domain to phosphoinositides, G protein subunits, or the FERM domain of Fak. These interactions lead to conformational changes of Tec and translocation to the cytoplasmic membrane where, in a second step, Src kinases phosphorylate a conserved tyrosine residue in the catalytic domain thereby increasing Tec kinase activity. Autophosphorylation of a tyrosine residue in the SH3 domain further prevents the inhibitory intramolecular interaction resulting in a robust Tec kinase activation.

Among the substrates and downstream effectors of Tec kinases are phospholipase $Cy2$ and PKC βI resulting in a sustained calcium influx and eventual activation of MAPKs. The interaction with the GDP/GTP exchange factor Vav can potentially activate Rac/Cdc42/Rho pathways, which can modulate actin cytoskeleton dynamics and also lead to JNK and p38 MAPK activation eventually inducing apoptosis. Paradoxically, Tec kinases may also trigger antiapoptotic signals by stimulating PI3-kinase and ▶Akt and promote proliferation by activating STATs.

Naturally occurring mutations of Btk were identified in human immunodeficiency diseases and X-linked agammaglobulinemia, where a lack of mature circulating B cells and immunoglobulins are observed, supporting a central role for Btk in B-cell maturation. In contrast, the knockout of Itk results in a lack of mature T cells and defects in T-cell receptor signaling. Furthermore, Btk and Etk/Bmx are able to complement a weakly oncogenic Src

in transformation of hepatocytes and fibroblasts suggesting their participation in anchorage-independent growth and development of cancer.

Syk

Syk and ZAP-70 are early intermediates in the transduction of signals from immune receptors, including the B- and T-cell receptors for antigen, activatory natural killer-cell receptors, the mast cell and basophil receptor for IgE, and the widely distributed receptors for the Fc portion of IgG. Immune receptors control checkpoints in lymphocyte development and serve to integrate the responses of innate and acquired immunity.

The current model proposes that upon engagement of immune receptors Src-family kinases are recruited that phosphorylate tyrosine residues in specific regions of the receptors, the immunoreceptor tyrosine-based activation motifs (ITAMs). These phosphotyrosines serve as docking sites for the SH2 domains of Syk and ZAP-70 that subsequently autophosphorylate and generate binding sites for SH2 domain containing proteins, like phospholipase $C\gamma$, Vav, and the adaptor protein Cbl. Furthermore, Syk and ZAP-70 phosphorylate a number of cytosolic and transmembrane linker proteins, such as SLP-76 (SH2-containing leukocyte protein of 76 kD), LAT (transmembrane linker for activation of T cells), TRIMM (T-cell receptor-interacting molecule), and SIT (SHP2-interacting transmembrane adaptor protein) that function as scaffolds to localize and assemble signaling complexes. In addition to being a major player in immune receptor signaling, Syk has a role in the “inside-out” integrin activation signal that is necessary for fibrinogen binding and subsequent aggregation of platelets during hemostasis.

Drugs

PTKs have been shown to play significant roles in the development of many disease states, including immunodeficiency, atherosclerosis, psoriasis, osteoporosis, diabetes, and cancer. Therefore, in the last years numerous PTK inhibitors have been developed that are currently undergoing clinical trials. The majority of them are directed against RTKs; however, most impressive clinical results have been obtained with Imatinib (STI-571, signal transduction inhibitor 571, CGP57148B, *Glivec*TM, *Gleevec*TM) that in addition to its action against PDGF and c-Kit receptors, potently inhibits the activity of the nonreceptor PTK c-Abl. Imatinib is a phenylamino-pyrimidine that, like almost all kinase inhibitors, competes for binding of ATP to the catalytic domain. However, the mechanism of action of Imatinib is unique: it binds to and stabilizes an inactive conformation of the kinase domain preventing activation of c-Abl. In CML as well as in a subset of ALL Bcr-Abl fusion proteins with elevated kinase activity are the

causative molecular abnormalities. In clinical trials with CML patients, impressive response rates of more than 90% without severe adverse effects have been observed with once-daily oral doses of Imatinib. However, more recently a cardiotoxic potential of Imatinib was described and the impact of this observation on the therapeutic use of Imatinib is not yet foreseeable. As with almost all chemotherapeutics, resistance to Imatinib as a single agent was observed in patients that were irresponsive or relapsed after initial successful treatment. Major mechanisms for this resistance are Bcr-Abl amplifications and mutations in the kinase domain that reduce affinity for the drug. Imatinib resistance is addressed by second-generation c-Abl inhibitors, such as INNO-406 (NS-187, CNS-9), Nilotinib (AMN-107, *Tasigna*TM) and Dasatinib (BMS-354825, *Sprycel*TM) that has been approved for CML in 2006. Meanwhile, Imatinib is also used for the treatment of gastrointestinal stroma tumors (GIST), dermatofibrosarcoma protuberans (DFSP), myelodysplastic/myeloproliferative diseases (MDS/MPD), aggressive systemic mastocytosis (ASM), and hypereosinophilic syndrome/chronic eosinophilic leukemia (HES/CEL). The clinical activity of Imatinib in GIST is based on its ability to inhibit c-Kit, which is frequently mutated in this disease. Other indications, such as DFSP, HES/CEL, and a certain proportion of CML involve aberrant PDGF receptor signaling that is blocked by Imatinib.

Several other inhibitors of nonreceptor PTKs are currently in development but only a few of them are studied in clinical trials. Noteworthy, Dasatinib does not only inhibit c-Abl, but also potently blocks Src activity, a property that may contribute to its beneficial clinical effects in CML. Other kinase inhibitors being developed that inhibit c-Abl and/or Src are AZD-0530, AP-23994, PD-0183805, SU-6656, and Bosutinib (SKI-606). Furthermore, peptidomimetic SH2 domain inhibitors for Src, such as AP-22408 have been designed that decrease bone resorption and may be promising drugs to treat osteoporosis and other bone diseases, such as Paget's disease and osteolytic bone metastasis.

Other experimental drugs against nonreceptor PTKs with therapeutic potential are Jak2 inhibitors, such as AG490, a benzenemalononitrile that was shown to suppress the growth of leukemic cells and ovarian as well as breast cancer cell lines, to reduce myocardial infarct size and cardiomyocyte apoptosis in ischemia/reperfusion injury and to prevent experimental allergic encephalomyelitis. Furthermore, Fak and several Src family kinases are considered as valid targets for an anticancer therapy.

Future basic research and clinical studies will show whether other inhibitors of nonreceptor PTKs may be as successful drugs as Imatinib for the treatment of fatal diseases such as cancer.

- Table Appendix: Protein Kinases
- Growth Factors
- Hematopoietic Growth Factors
- JAK-STAT Pathway
- Neurotrophic Factors

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Tyrosine Phosphatases

In contrast to tyrosine kinases, Tyrosine phosphatases (PTPs) are enzymes which act on phosphorylated proteins and catalyze the transfer of a phosphate group from a tyrosine residue to a water molecule, generating orthophosphates in a process which is referred to as “dephosphorylation.” PTPs are involved in many cellular signal transduction pathways.

- Growth Factors
- Tyrosine Kinases

Tyrosylprotein Kinase

- Tyrosine Kinases

Tyrphostins

Tyrphostins are a group of substances, which block a variety of tyrosine kinases. Some of them have a relative selectivity for defined tyrosine kinase subtypes.

- Tyrosin Kinases

Ubiquinone

► Coenzyme Q₁₀

Ubiquitin-Dependent Protein Degradation

Ubiquitin tags proteins for protein degradation. The ubiquitination requires three different enzymatic activities, a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2 or Ubc) and a ubiquitin ligase (E3). The action of all three enzymes leads to the establishment of a poly-ubiquitin chain on target proteins which are then recognized and proteolyzed by the 26S proteasome.

► Ubiquitin/Proteasome

Ubiquitin/Proteasome

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Synonyms

Protein degradation; Posttranslational protein modification

Definition

Protein modification by the covalent attachment of ubiquitin chains serves as a signal to mark proteins for the degradation by a multicatalytic proteinase complex called the proteasome. Thus the ubiquitin proteasome system (UPS) controls the stability of proteins in a

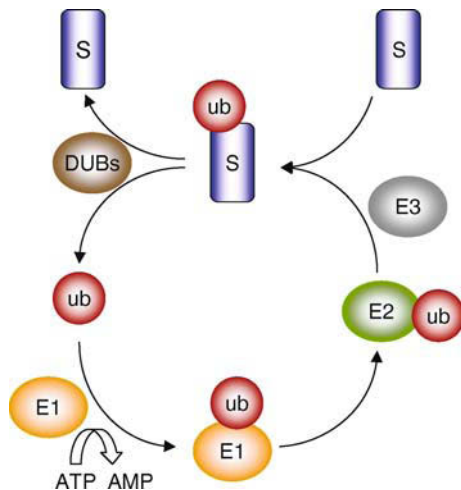
regulated manner affecting multiple essential cellular processes. In addition, dependent on the mode of linkage, ubiquitin regulates protein-protein interaction, endocytosis, replication and the formation of signaling complexes in a proteasome-independent fashion. Beside phosphorylation ubiquitination represents the most important posttranslational regulatory mechanism in biology.

Basic Mechanisms Ubiquitin Conjugation

Ubiquitin is a highly conserved 8.5 kDa polypeptide, which was first described in 1974. The discovery that the Ubiquitin proteasome system serves as a general mechanism to target proteins for destruction by the proteasome was awarded with the Nobel Prize for Chemistry in 2004.

Upon cleavage from a precursor ubiquitin becomes covalently attached to the ε-amino group of lysine (K) residues in specific target proteins by the consecutive activity of a set of E1, E2 and E3 enzymes. First an ubiquitin-activating enzyme (E1) is charged with ubiquitin. This is accomplished by using ATP to form a high energy bond at the C-terminus of ubiquitin. Subsequently this linkage is attacked by the sulfhydryl group of the active site cysteine present in the catalytic domain of the E1 enzyme resulting in a thioester formed between the E1 and ubiquitin. Ubiquitin can now be transferred from the E1 to the catalytic core of a second class of enzymes termed ubiquitin-conjugating enzymes (E2). The E1 is released and can now charge additional ubiquitin residues. Ubiquitin bound to E2 can finally be transferred to target protein substrates, a step which is catalyzed by E3 ligases. As ubiquitin itself contains several lysine residues, which can serve as targets for the linkage of additional ubiquitin molecules, variable chains of ubiquitin can be attached. Like most posttranslational modification mechanisms ubiquitin conjugation is a reversible process. Analogous to the activity of phosphatases in protein phosphorylation, ► **deubiquitinating enzymes (DUBs)** counteract ubiquitin modification by cleaving the molecule from modified substrates.

The hierarchical order of E1, E2 and E3 activity correlates with an increase in enzymatic specificity which ensures selective ubiquitin modification of distinct



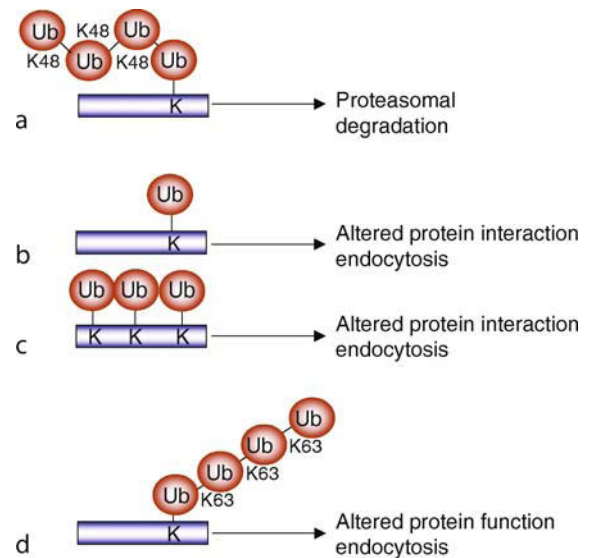
Ubiquitin/Proteasome. Figure 1 Ubiquitin conjugation cycle. Ubiquitin (Ub) is ligated to target substrates (S) by the consecutive activity of ubiquitin activating enzymes (E1), Ubiquitin conjugating enzymes (E2) and ubiquitin ligases (E3). Deubiquitinating enzymes (DUBs) mediate deconjugation of ubiquitin bound to substrates.

target proteins. While only two E1 enzymes and around 50 E2 enzymes have been described more than 700 E3s are encoded in the human genome. The large group of E3 enzymes is mainly responsible to exert specificity in terms of substrate recognition. The existence of more than 80 different DUBs in humans adds further complexity to the system (Fig. 1).

Ubiquitin Modification as a Signal for Proteasomal Degradation

Single ubiquitin residues can be connected to one (monoubiquitination) or multiple lysine residues (multi-monoubiquitination) of the target protein. As different lysines within ubiquitin can serve as sites for the addition of further ubiquitin molecules, variable chains of ubiquitin can be connected to the substrate (polyubiquitination). The best-characterized lysines used for ubiquitin chain prolongation are K48 and K63. As depicted in Fig. 2 the fate of ubiquitin labeled proteins depends on the mode of linkage that is formed [1].

K48-linked polyubiquitin serves as a signal for proteolytic degradation by the 26S proteasome, a multisubunit complex composed of a core protease termed 20S proteasome and a 19S regulatory particle (PA700), attached at either one or both ends. The 19S regulatory particle can further be dissected into two substructures, a lid and a base. The base harbors six homologous ATPases. The 20S proteasome consists of four heptameric rings ($\alpha 1$, $\beta 1$, $\beta 2$, $\alpha 2$) that form a cylindric, barrel shaped structure harboring three distinct catalytic domains in the β -subunits orientated towards the internal side. These different catalytic subunits were

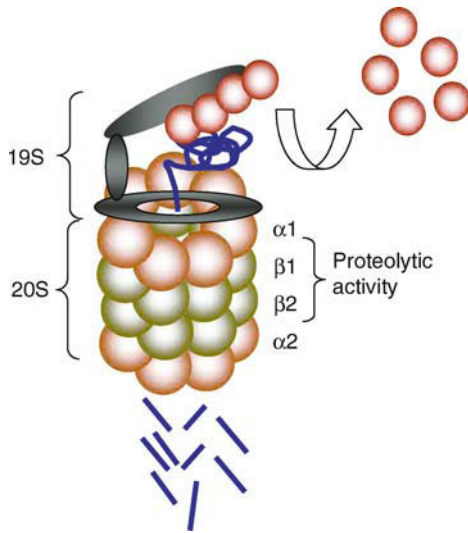


Ubiquitin/Proteasome. Figure 2 Functional consequences of ubiquitin linkage. Substrates (blue bars) are linked via lysine residues (K) to ubiquitin or ubiquitin chains. (a) Attachment of chains connected via Lysines in position 48 of ubiquitin (K48) targets substrates for proteasomal degradation. In contrast modification of one (b) or multiple (c) lysines by a single ubiquitin molecule mediates novel protein interactions or initiates endocytosis. Conjugation of K63-linked polyubiquitin (d) alters protein function and can also serve as a signal for endocytosis.

shown to encompass chymotrypsin-like, trypsin-like and peptidyl-hydrolase like activity.

Recognition of ubiquitin conjugated proteins, detachment of the ubiquitin chains, and unfolding is accomplished by the regulatory 19S subunit. From there the polypeptide is translocated in the lumen of the 20S core proteasome and degraded by the proteolytic activity of the β -subunits (Fig. 3). The pore can be occluded by peptides from the α -subunits and PA700 functions as a gatekeeper by removing these occlusions. The process of 26S proteasome-catalyzed proteolysis is energy dependant i.e. depends on ATP hydrolysis.

The modular structure allows adaptation to different physiological demands by the exchange of subunit components in either the regulatory or proteolytic part of the proteasome. IFN- γ for example can modify the composition of the proteasome by mediating the exchange of the regulatory complex and replacement of proteolytic subunits. Proteolytic activities in this so-called immunoproteasome are altered in a way that generation of antigenic peptides suitable to be loaded to **MHC class I proteins** is favored. Thus the ubiquitin proteasome system exerts an essential function in antigen presentation, necessary for appropriate immune defense against pathogens [2].



Ubiquitin/Proteasome. Figure 3 Structure of the proteasome. Proteasomes form a barrel like structure and are composed of a 20S core particle and the 19S regulatory subunit located at one or both ends. Two heptameric β -rings (green), which contain the proteolytic domains, are attached to two outer α -rings (orange). Substrates (blue) are recognized by the regulatory particle, unfolded and upon detachment of ubiquitin chains (red) degraded in the lumen of the 20S core proteasome.

The UPS also plays a major role in protein quality control. In a process known as endoplasmic associated degradation (ERAD), misfolded proteins, which are formed in the endoplasmic reticulum, are translocated back to the cytoplasm and degraded by the proteasome.

The ubiquitin mediated degradation of certain proteins is also one of the key mechanisms underlying cellular growth control. Thus the half life of critical regulatory proteins of the cell cycle like p53, cyclins and cyclin dependant kinases (CDKs) is directly controlled by the ubiquitin system. Furthermore cell cycle progression depends on a coordinated network of ubiquitin mediated proteolysis of these factors. Key regulators of cell cycle checkpoints and cyclin degradation are the ►anaphase promoting complex (APC) and the ►Skp1/Cul1/F-box protein complex (SCF) both acting as ubiquitin ligases.

APC is active from mid-M phase (anaphase) to the end of G1 phase and required for disconnecting sister chromatids and exit from M-Phase to G1. The complex mediates the ubiquitination of Securin and Cyclin B. Degradation of these proteins, which block mitotic progression, promotes anaphase onset and exit from mitosis.

The SCF ubiquitylates proteins from late G1 to early M phase. This complex consists of a core together with different so called F-box proteins, which are responsible for the substrate recognition. Typically

interaction of F-box proteins with their substrate involves sites of phosphorylation, so called phosphodegrons. Thus a highly coordinated and specific degradation of different cell cycle regulators in distinct activation stages can be achieved [3].

Proteasomal degradation also plays an essential role in the activation of cellular signaling pathways. A prototype for this is the control of NF- κ B signaling, which has a pivotal role in inflammatory responses. Upon stimulation the inhibitory I κ B α protein is phosphorylated and thereby becomes a target substrate for K48-polyubiquitination. Proteasomal degradation of I κ B α releases the transcription factor NF- κ B, which subsequently translocates to the nucleus and activates specific target genes.

Non-Proteolytic Functions of Ubiquitin Conjugation

In contrast to the proteasome mediated degradation of proteins linked to K48-polyubiquitin chains, monoubiquitin-, multi-monoubiquitin- or K63-linkage of ubiquitin chains is not a signal for proteasomal degradation, but controls molecular mechanisms like protein activity and interaction, DNA repair and inflammation. Mono-ubiquitination and multi-monoubiquitination are crucial events in signal termination. Upon ligand binding surface receptors like receptor tyrosine kinases or G-protein coupled receptors are hereby marked for endocytosis and degraded via the endosomal/lysosomal pathway. Another example for non-proteolytic functions is the modification of proliferative cell nuclear antigen (PCNA), a DNA processivity factor. While K63-linked polyubiquitin modification results in error-free DNA repair, mono-ubiquitination of PCNA recruits an error-prone ►translesion polymerase [4].

Ubiquitin modification of substrates can be sensed by proteins, which serve as “ubiquitin receptors.” These proteins harbor domains capable of ubiquitin binding and help to translate the signal into the proper physiological response by forming signaling complexes or activating downstream effectors. So far more than 15 different ubiquitin recognition motifs have been identified.

Ubiquitin Like Proteins

Proteins can also be modified by so-called ubiquitin-like modifiers (UBLs). These proteins have structural similarity to ubiquitin and are linked to substrates in a similar way. Like ubiquitin UBLs are conjugated by the consecutive action of E1, E2 and E3 ligases and sometimes use the same enzymes mediating ubiquitin linkage. Prominent examples for UBLs are ►SUMO, ►NEDD8, ►FAT10 or ►Interferon stimulated gene 15 (ISG15), which have been shown to affect multiple cellular functions like protein localization, cell cycle progression, DNA repair and immune function [5].

Disease Associated with Perturbations of the Ubiquitin System

In concordance with the central role of ubiquitin modification in multiple cellular functions perturbations of this system are associated with a variety of diseases. Defects in the control of cell cycle regulators by the ubiquitin proteasome system are connected to cancer progression and many E3 ligases were originally identified as oncogenes.

As the ubiquitin proteasome pathway is a main route for protein clearance it is not surprising that in proteinopathies (disease caused by aggregate prone proteins) like sporadic Parkinson- or Huntington disease proteasome activity is reduced. Autosomal recessive loss of function of the E3 ligase parkin is the molecular base for one of the most common forms of familial Parkinson disease.

In ►**Cystic fibrosis** a point mutation of the cystic fibrosis transmembrane regulator (CTFR) prevents transport of this molecule to the cell surface. Instead this otherwise functional molecule is degraded by the ERAD-ubiquitin proteasome pathway.

Pharmacological Intervention

Proteasomal inhibition represents a novel strategy in cancer treatment and the small molecule Bortezomid (PS-341, Velcade™) has been approved for the treatment of refractory and relapsed multiple myeloma, a proliferative disease of plasma cells. Bortezomid inhibits an active site in a proteasome subunit and remarkably shows selective cytotoxicity to cancer cells. Although the underlying mechanisms are not completely understood bortezomid apparently induces a cell stress response in these tumor cells followed by caspase-dependent apoptosis. Whether bortezomid is beneficial for the treatment of other proliferative disease is currently being tested in clinical trials.

An example for a specific pharmacological approach within the ubiquitin system is to prevent ubiquitin mediated degradation of the tumor suppressor p53, which is mediated by the E3 ligase HDM2. Inhibition of HDM2 ligase activity by a family of closely related 7-nitro-5 deazaflavin compounds (HLI98) was shown to stabilize p53. Protein interaction between HDM2 and p53 was effectively prevented by nutlins, a class of *cis*-imidazoline compounds, which exhibited strong anti tumor effects in mice [3, 5].

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Ubl1

Synonym

Ubiquitin-like Protein 1

►SUMOylation

UDP-Glucuronyl Transferase

UDP-glucuronyl transferases (UGTs) are a group of enzymes which catalyze the transfer of UDP-glucuronyl moieties from UDP-glucuronic acid into a variety of small lipophilic agents, which can be xenobiotics (drugs, environmental toxicants, carcinogens), as well as endogenous substances (steroids, bile acids, bilirubin, hormones, dietary constituents). Genes encoding UGTs have been cloned, and there are at least 15 UGTs in the mammalian system. Genetic polymorphisms have been observed in a variety of UGTs, which are responsible for differences in drug metabolism as well as for diseases like Crigler-Najjar's and Gilbert's syndrome.

Ultrarapid Metabolizer

An ultrarapid metabolizer (UM) is a drug metabolism phenotype that describes the ability to metabolize a drug at much faster rates than expected. The term was originally created for individuals who carry an allele of CYP2D6 with two or more functional gene copies, which results in increased enzyme protein being expressed in the liver. This condition can lead to lack of response and therapeutic failure, e.g. during treatment with antidepressants that are CYP2D6 substrates. Reliable prediction of the UM phenotype is not possible based on the genotype alone but requires phenotype determination using a probe drug.

►P450 Mono-oxygenase System

Unstable Angina

Defined as worsening (in intensity or frequency) angina attacks, sudden-onset angina at rest, and angina lasting more than 15 min. As these may herald myocardial infarction, they require urgent medical attention.

► Atherosclerosis

Unwanted Effects

► Adverse/Unwanted Reaction

UPR

Unfolded protein response, when mis-folded proteins accumulate in the ER, signal transduction pathways are activated that increase the biosynthetic capacity and decrease the biosynthetic burden of the ER.

► Chaperones

Uptake Transporter

While ABC-transporters eliminate their substrates in an energy-dependent manner from the intracellular space, uptake transporter facilitate the uptake of several substances into the cell. Currently a lot of different uptake transporters have been identified; they are organized in the so called solute carrier superfamily (SLC), which consists of around 350 members. While most of these transporters are not involved in the transport of pharmacological agents, members of the SLC22A- and SLCO-family have been demonstrate to be important in this context. The SLC22A-family comprises transporters for organic cations (organic cation transporter, OCT) as well as anions (organic anion transporter, OAT), while the members of the SLCO-family are especially involved in the transport of

bulky organic anions. In contrast to ABC-transporter, which generate their driving force by hydrolysis of ATP, the SLC-transporter act as sym- or antiporters and facilitate the uptake into the cell by building up a pore.

► ABC Transporter

Urate

► Uric Acid

Urea Transporter

UT1, the urea transporter, is an integral membrane protein which is highly selective for urea and is expressed at the apical plasma membrane of principal cells of the collecting duct. The apical plasma membrane is the rate-limiting membrane for overall transcellular urea transport. The UT1 is activated by vasopressin through the V2 receptor, urea is then transported through the cell into the interstitium. In this way urea contributes to the corticocapillary osmolality gradient, which provides the driving force for water reabsorption in the inner medulla of the kidney.

► Vasopressin/Oxytocin

► Anti-gout Drugs

Uric Acid

Uric acid is the endproduct of purine metabolism in man. Uric acid has a lower solubility than its progenitor metabolites, hypoxanthine and xanthine. Impaired uric acid elimination and/or increased uric acid production result in hyperuricemia and increase the risk of gouty arthritis. At physiological pH, 99% of the uric acid molecules are actually in the form of the urate salt. A decrease in pH increases the fraction of uric acid molecules relative to urate molecules. Uric acid possesses lower solubility than urate.

► Anti-gout Drugs

Uricostatic Drug

Uricostatic drugs inhibit the production of uric acid through the inhibition of xanthine oxidase. Allopurinol is the only therapeutically used uricostatic drug.

► Anti-gout Drugs

Uricosuric Drug

Uricosuric drugs increase the renal excretion of uric acid by inhibiting its renal reabsorption. Therapeutically used uricosuric drugs are benzbromarone, probenecid and sulfinpyrazone.

► Anti-gout Drugs

Urodilatin

Urodilatin is a peptide similar to atrial natriuretic peptide, which is produced in the distal tubule of the kidney and promotes sodium excretion and diuresis by acting on receptors localized on the luminal site of the collecting duct of the nephron.

► Guanylyl Cyclases

Urographic Contrast Agents

Urographic contrast agents are contrast agents which possess the characteristics of very little enteral absorption, almost no protein binding or uptake into cells, an extracellular (interstitial) distribution and glomerular filtration. These pharmacokinetics are due to very little interaction with the organism, resulting in very low toxicity, preferably nonionic (neutral) molecules.

► Radiocontrast Agents

Urokinase-Type Plasminogen Activator

Urokinase-type plasminogen activator (uPA, urokinase) is synthesized by endothelial and tumor cells as a single-chain glycoprotein (scuPA) without catalytic activity. When it is converted to a two-chain protein (tcuPA) by plasmin, an active serine protease center develops, which activates plasminogen. Thus, uPA (55 kDa) results in the amplification of fibrinolysis.

► Fibrinolytics

Urotensin

Urotensin is a cyclic peptide of 11 amino acids, cleaved from a larger prepro-urotensin II precursor peptide of about 130 amino acids. The cyclic region of the peptide, which confers biological activity, has been highly conserved in evolution from fish to mammals. In humans, prepro-urotensin II is expressed mainly in the brain and spinal cord; it is also detected in other tissues, such as kidney, spleen, small intestine, thymus, prostate, pituitary and adrenal gland. In contrast, the receptor for urotensin II is found predominately in the heart and arterial vessels. Urotensin II is the most potent mammalian vasoconstrictor yet identified, being of the order of 10-fold more potent than endothelin-1. The receptor of urotensin II has recently been identified (GPR14) and belongs to the group of G-protein-coupled receptors.

Urticaria

Urticaria is a usually transient skin reaction marked by edema and the formation of wheals, smooth, raised areas.

- Allergy
- Antiplatelet Drugs
- Hematopoietic Growth Factors
- Histaminergic System

UT1

► Urea Transporter

V-type ATPase

► Table appendix: Membrane Transport Proteins

similar functional properties, there are some differences in subunit composition.

► Vesicular Transporters

Vaccination

► DNA Vaccination and Genetic Vaccination

VACht

Vesicular acetylcholine transporters.

► Vesicular Transporters

Vacuolar-type Proton Translocating ATPase

Vacuolar-type proton translocating ATPase is a heteromeric protein complex, which appears to translocate two protons across the vesicle membrane for each ATP molecule that is hydrolyzed, generating chemical (ΔpH) and electrical ($\Delta\Psi$) gradients. Although the ATPases present on different classes of intracellular vesicle have

Vanilloid Receptor

The vanilloid receptor is named for its ability to respond to molecules that contain a vanillyl moiety, such as capsaicin, the “hot” component of chili peppers or resiniferatoxin. The vanilloid receptor mediates the pain evoked by capsaicin. It has been shown to be an ion channel, which belongs to the group of “transient-receptor-potential” (TRP) cation channels. Together with at least four other TRP-channels, it forms the subgroup of TRPV channels. TRPV1 (the vanilloid receptor; VR1) is mainly expressed in the sensory nerves and is activated physiologically by heat ($> 43^\circ\text{C}$), anandamide, phosphatidylinositol 4,5-bisphosphate (PIP_2) and H^+ ions.

► TRP Channels

Varizella Zoster Virus

Varizella zoster virus (VZV) is a highly contagious herpesvirus causing chickenpox upon primary infection. After recovery, the virus stays dormant in nerve roots. Weakening of the immune system, e.g. in people over the age of 60 or under immunosuppressive therapy, can lead to reactivation of VZV. This recurrence causes shingles (herpes zoster), a painful rash that develops in a well-defined band corresponding to the area innervated by the affected nerve cells.

► Antiviral Drugs

Vascular Endothelial Growth Factor

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Definition

► **Angiogenesis** is known to be fundamental to a variety of physiological processes including embryonic and postnatal development, reproductive functions and wound healing. Furthermore, neovascularization plays an important pathogenic role in tumorigenesis and in the vision loss associated with ischemic retinal disorders and the wet form of age-related macular degeneration (AMD). Research performed in recent decades has established that angiogenesis is a complex and coordinated process, which requires a series of signaling steps in endothelial and mural cells elicited by numerous families of ligands. Moreover, a variety of endogenous inhibitors of angiogenesis have been identified, including endostatin, tumstatin and vasostatin. However, despite such complexity and potential redundancy, ► **vascular endothelial growth factor** (VEGF)-A appears to be necessary for growth of blood vessels in a variety of normal and pathological circumstances. *VEGF-A* is the prototype member of a gene family that includes also placenta growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D and the orf-virus encoded VEGF-E.

Basic Characteristics

VEGF-A stimulates the growth of vascular endothelial cells derived from arteries, veins and. VEGF-A induces angiogenesis in a variety of in vivo models. Inactivation of a single VEGF-A allele results in embryonic lethality, indicating that during early development there is a critical VEGF-A gene-dosage requirement. VEGF-A plays an important role also in early postnatal life. Administration of VEGF inhibitors results in growth arrest and lethality in mice when the treatment is initiated at day 1 or day 8 postnatally. VEGF is important for endochondral bone formation and growth plate angiogenesis and morphogenesis. VEGF-A blockade reversibly inhibits of skeletal growth. Another key function of VEGF-A is the regulation of the cyclical angiogenesis that occurs in the female reproductive tract.

Alternative exon splicing results in the generation of four main VEGF-A isoforms, which have respectively 121, 165, 189 and 206 amino acids after the signal sequence is cleaved (VEGF₁₂₁, VEGF₁₆₅, VEGF₁₈₉, VEGF₂₀₆). Less frequent splice variants have also been reported (reviewed in [1]).

Like VEGF₁₆₅, native VEGF is a heparin-binding homodimeric glycoprotein of 45 kDa. In contrast, VEGF₁₂₁ lacks heparin-binding properties. VEGF₁₈₉

and VEGF₂₀₆ bind to heparin with affinity comparable to that of bFGF. Whereas VEGF₁₂₁ is a freely diffusible protein, VEGF₁₈₉ and VEGF₂₀₆ are almost completely bound to heparin-like moieties in the cell surface or in the extracellular matrix. VEGF₁₆₅ has intermediate properties in terms of heparin-affinity and bioavailability. The long isoforms may be released in a diffusible form by proteolytic cleavage. Early studies showed that plasmin is able to cleave VEGF₁₆₅ at the COOH terminus, generating VEGF₁₁₀, a bioactive fragment consisting of the first 110 NH₂-terminal amino acids. Recent studies have shown that various matrix metalloproteinases (MMPs) -especially MMP-3- may also cleave VEGF₁₆₅ to generate diffusible, non-heparin binding fragments.

VEGF-A binds two highly related ► **receptor tyrosine kinases** (RTK), VEGFR-1 and VEGFR-2. VEGFR-1 was the first RTK to be identified as a VEGF receptor more than a decade ago, but the precise function of this molecule is still debated in the field [1]. The functions and signaling properties of VEGFR-1 appear to vary with the developmental stage and the cell type, e.g. endothelial versus non-endothelial cells. VEGFR-1 binds not only VEGF-A but also PlGF and VEGF-B and fails to mediate a strong mitogenic signal in endothelial cells. Non-mitogenic functions mediated by VEGFR-1 in the vascular ► **endothelium** include the release of growth factors and the induction of MMP-9. Furthermore, VEGFR-1 mediates hematopoiesis and monocyte chemotaxis in response to VEGF-A or PlGF.

VEGFR-2 also binds VEGF-A with high VEGF-C and VEGF-D may also bind and activate VEGFR-2, following their proteolytic cleavage. The key role of VEGFR-2 in developmental angiogenesis and hematopoiesis is underscored by lack of vasculogenesis and failure to develop blood islands and organized blood vessels in Flk-1 null mice. There is now agreement that VEGFR-2 is the major mediator of the angiogenic and permeability-enhancing effects of VEGF-A.

Neuropilin-1 (NRP1), a molecule that had been previously shown to be implicated in axon guidance as a receptor for members of collapsin/semaphorin family, has been characterized as a which interacts with the heparin-binding VEGF isoforms.

Drugs

Many tumor cell lines secrete VEGF-A in vitro (reviewed in [1]) In situ hybridization studies have demonstrated that the VEGF mRNA is expressed in many human tumors A variety of transforming events also lead to induction of VEGF gene expression. Oncogenic mutations or amplification of ras lead to VEGF up-regulation. Renal cell carcinomas have a particularly high level of VEGF-A expression, consistent with the notion that inactivating mutation in the von Hippel-Lindau (VHL) tumor suppressor gene, resulting in high transcription of the hypoxia-inducible factor

(HIF)-target genes under normoxic conditions, occur in ~50% of such tumors.

Monoclonal antibodies targeting VEGF-A have been reported to inhibit the growth of several tumor cell lines in nude mice. Inhibition of tumor growth has been achieved also with other anti-VEGF-A treatments, including small molecule inhibitors of VEGFR-2 signaling (reviewed in [2]), anti-VEGFR-2 antibodies.

Although tumor cells frequently represent the major source of VEGF-A, tumor-associated stroma is also a site of VEGF production. Tumor-derived PDGF-A may be especially important for the recruitment of an angiogenic stroma that produces VEGF-A and potentially other angiogenic factors.

Combining anti-VEGF treatment with chemotherapy or radiation therapy results in a greater anti-tumor effect than either of these therapies alone.

Several VEGF inhibitors have been developed as anti-cancer agents. These include a humanized anti-VEGF-A monoclonal antibody (bevacizumab), an anti-VEGFR-2 antibody, various small molecules inhibiting VEGFR-2 signal transduction [2], and a VEGF receptor chimeric protein.

The clinical trial that resulted in FDA approval of bevacizumab (February 2004) was a randomized, double-blind, phase III study in which bevacizumab was administered in combination with bolus-IFL (irinotecan, 5FU, leucovorin) chemotherapy as first-line therapy for previously untreated metastatic colorectal cancer [3]. Median survival was increased from 15.6 months in the bolus-IFL + placebo arm to 20.3 months in the bolus-IFL + bevacizumab arm.

The clinical benefit of bevacizumab is being evaluated in a broad variety of tumor types and lines of therapy including combination studies with several biologicals. Bevacizumab, combined with weekly paclitaxel in women with previously untreated metastatic breast cancer provided a significant improvement in the primary endpoint of progression free survival relative to paclitaxel alone. Combining bevacizumab with paclitaxel and carboplatin in patients with previously untreated, nonsquamous, NSCLC provided a significant improvement in the primary endpoint of overall survival. Also, combining bevacizumab with 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) in patients with previously treated metastatic colorectal cancers provided a significant improvement in the primary endpoint of survival.

Preliminary, encouraging data with bevacizumab have been reported in a number of other cancers including renal cell ovarian and prostate. Taken together, these findings suggest that targeting VEGF-A with a neutralizing antibody may be a broadly applicable approach to the treatment of human cancer.

Besides bevacizumab, several other types of VEGF inhibitors are being developed. Among these, a variety of small molecule RTK inhibitors targeting the VEGF

receptors are at different stages of clinical development. The most advanced are SU11248 (sunitinib) and Bay 43-9006 (nexavar). SU11248 inhibits tyrosine phosphorylation of VEGFRs, platelet-derived growth factor receptors (PDGFRs), c-kit and Flt-3 and has shown efficacy in imatinib-resistant gastrointestinal stromal tumor and renal cell carcinoma. SU11248 was FDA-approved for the treatment of imatinib-resistant gastrointestinal stromal tumor. Also, Bay 43-9006 results in a significant increase in progression-free survival in patients with advanced renal cell carcinoma and the drug has been approved by the FDA for this indication.

Age-related macular degeneration (AMD) is the most common cause of severe, irreversible vision loss in the elderly. AMD is classified as nonexudative (dry) or exudative (wet or neovascular) disease. Although the exudative form accounts for ~10–20% of cases, it is responsible for 80–90% of the visual loss associated with AMD. Three pharmacologic therapies for neovascular AMD have been approved by the FDA: verteporfin (Visudyne[®]) photodynamic therapy (PDT), pegaptanib and ranibizumab [4]. Pegaptanib sodium is a pegylated oligonucleotide aptamer that binds to and inactivates VEGF₁₆₅. Pegaptanib sodium prevented moderate vision loss in 70% of subjects compared with 55% for the control group at one year. However, on average, patients in the pegaptanib sodium group lost ~8 letters at one year, compared with a loss of ~15 letters in the sham injection group. Ranibizumab is a recombinant, humanized Fab that binds to and potentially neutralizes the biological activities of all known human VEGF-A isoforms, as well as the proteolytic cleavage product VEGF₁₁₀. Pivotal trial evaluating the safety and efficacy of ranibizumab in randomized subjects with wet AMD to monthly sham injections or monthly intravitreal injections of one of two doses of ranibizumab have demonstrated a significant benefit, including increases in visual acuity, in approximately one third of the patients treated with ranibizumab.

Perspectives

Research conducted for almost two decades has established that VEGF-A is important for regulation of the normal angiogenesis processes. Moreover, VEGF inhibition has been shown to suppress pathological angiogenesis in a variety of cancer models, leading to the clinical development of a variety of VEGF inhibitors. Definitive clinical studies have proved that VEGF inhibition, by means of bevacizumab in combination with chemotherapy, provides a significant clinical benefit, including increased survival, in patients with previously untreated metastatic colorectal cancer [3]. Furthermore, SU11248 and Bay 43-9006 have been recently approved by the FDA for metastatic renal cell carcinoma and their mechanism of tumor suppression consists, at least partly, of inhibition of VEGF signaling.

A particularly active area of research concerns the elucidation of the mechanisms of refractoriness or resistance to anti-VEGF therapy. Tumor cell-intrinsic or treatment-induced expression of angiogenic factors may be implicated. Very recent studies have provided evidence that, at least in some murine models, refractoriness to anti-VEGF therapy is related to the ability of the tumor to recruit CD11b+Gr1+ myeloid cells, which promote angiogenesis [5]. It remains to be established whether these findings also apply to human tumors.

Reliable markers are needed to monitor the activity of antiangiogenic drugs. Circulating endothelial cells and their progenitor subset are a potential candidate, as is MRI dynamic measurement of vascular permeability/flow in response to angiogenesis inhibitors, but neither has been clinically validated.

VEGF inhibitors have demonstrated a marked clinical benefit also in wet AMD. Blockade of all VEGF-A isoforms and bioactive fragments with ranibizumab not only slowed down vision loss, but unexpectedly appears to have the potential to enable many AMD patients to obtain a meaningful and sustained gain of vision. Further research is needed to determine whether the vision gain conferred by ranibizumab extends beyond 24 months and whether additional intraocular neovascular syndromes may benefit from this treatment.

- **Vascular Endothelial Growth Factor**
- **Angiogenesis**

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Vasoactive Intestinal Peptide

Vasoactive Intestinal Peptide (VIP) is a 28-amino acid peptide, which has a variety of actions as a neuroendocrine hormone and a putative neurotransmitter. It

stimulates prolactin secretion from the pituitary and catecholamine release from the adrenal medulla. It acts also on cells of the immune system, stimulates electrolyte secretion and relaxes smooth muscles. VIP is produced from a precursor polypeptide (prepro-VIP). Two G-protein coupled receptors have been described which mediate the actions of VIP, VPAC₁ (VIP₁, PACAP type II) and VPAC₂ (VIP₂, PACAP-3). Both receptors also respond to pituitary adenylate cyclase-activating polypeptide (PACAP) and are coupled via G_s in a stimulatory fashion to adenylyl cyclases. In the autonomic nervous system, VIP is part of the NANC transmitter system. It is typically coexpressed with acetylcholine in postganglionic parasympathetic neurons, and functions as a cotransmitter which is involved in the induction of vasodilatation or bronchodilatation.

- **Synaptic Transmission**
- **Cotransmission**

Vasoconstrictor

Vasoconstrictors are drugs which increase the tone of smooth muscle cells in the vasculature. They include sympathomimetic amines and certain eicosanoids and peptides (angiotensin, vasopressin, urotensin or endothelin).

- **Smooth Muscle Tone Regulation**

Vasodilator

Vasodilators are a group of drugs, which relax the smooth muscle cells of the blood vessels and lead to an increased local tissue blood flow, a reduced arterial pressure and a reduced central venous pressure. Vasodilators reduce the cardiac pre-load as well as after-load and thereby reduce cardiac work. They are used in a variety of conditions including hypertension, cardiac failure and treatment/prevention of angina pectoris. Major groups are Ca²⁺-channel blockers (e.g. dihydropyridines), NO-donators (e.g. organic nitrates), K⁺-channel openers (minoxidil), phosphodiesterase inhibitors (e.g. sildenafil), Rho-kinase inhibitors (e.g. Y27632) or substances with unknown mechanism of action (e.g. hydralazine). Inhibitors of the

renin-angiotensin-system (e.g. ACE inhibitors, angiotensin II receptor antagonists or renin inhibitors) also act as vasodilators.

► Smooth Muscle Tone Regulation

Vasopeptidase Inhibitors

Vasopeptidase inhibitors are a group of drugs (e.g., omapatrilat, sampatrilat) which have a dual mechanism of action in that they inhibit two metalloprotease enzymes, neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE). This results in increased availability of natriuretic peptides that exhibit vasodilatory effects (NEP-inhibition) and in reduced formation of angiotensin II (ACE-inhibition). Since these peptidases are intimately concerned with regulating the structural and functional properties of the heart and circulation, they were named “vasopeptidases.” Vasopeptidase inhibitors have been developed for the treatment of hypertension or congestive heart failure.

► Renin–Angiotensin–Aldosterone System

Vasopressin/Oxytocin

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Synonyms

Vasopressin: 8-arginine-vasopressin (AVP); Antidiuretic hormone (ADH); Lysipressin; Oxytocin (OT); 8-leucine-vasotocin

Definition

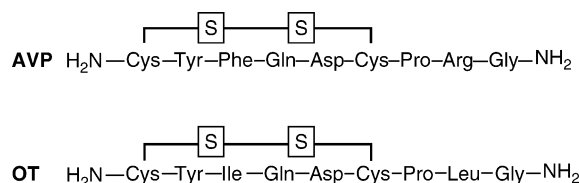
AVP plays a central role in water homeostasis of terrestrial mammals, leading to water conservation by the kidney. OT is primarily involved in milk ejection, parturition and in sexual and maternal behaviour. Both hormones are peptides secreted by the neurohypophysis, and both act also as neurotransmitters in the central nervous system (CNS). The major hormonal targets for AVP are the renal tubules and vascular myocytes. The hormonal targets for OT are the myoepithelial cells

that surround the alveolar channels in the mammary gland and the uterus. AVP plays a central role in pathological processes like ►diabetes insipidus (DI) and the syndrome of inappropriate antidiuretic hormone secretion (SIADH).

Basic Characteristics

AVP and OT are cyclic nonapeptides with a disulphide bridge between the cysteine residues 1 and 6, resulting in a six-amino acid ring and a COOH-terminal α -amidated three-residue tail. OT differs only in two amino acids from AVP: Ile in position 3, which is essential for OT receptor (OTR) stimulation and Leu in position 8. AVP has a Phe in position 3 and an Arg in position 8. Arg 8 is essential for acting upon vasopressin receptors (Fig. 1). Lysipressin, found in pigs and some marsupials, has a Lys in position 8 [1].

AVP and OT are synthesized initially as preprohormones consisting of a signal peptide, the hormone, the binding protein neurophysin and, only for vasopressin, the glycosylated peptide copeptin. The preprohormone is processed into its final products in neurons located with their cell bodies in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus. The axons of these cells project to form the neurohypophysis, where AVP and OT are secreted after appropriate stimuli. Two populations of neurons exist in the PVN: magnocellular neurons (with large cell bodies), terminating in the neurohypophysis, and parvocellular neurons (with smaller cell bodies), terminating in the median eminence, brain stem, spinal cord, limbic and olfactory areas. Parvocellular neurons terminating in the median eminence are the major source of hypophysiotropic corticotrophin releasing factor (CRF), which is released together with AVP (and possibly also OT) into the hypophysial portal circulation. High concentrations of CRF and AVP are transported by this route to the anterior pituitary gland, where they regulate the secretion of adrenocorticotrophic hormone (ACTH). A group of oxytocinergic neurons from the PVN project to extrahypothalamic brain areas (see below) and to the spinal cord, which are involved in the control of yawning, erectile function and copulation. Magnocellular and parvocellular neurons produce either AVP or OT [2].



Vasopressin/Oxytocin. Figure 1 Amino acid sequence of AVP and OT. The disulphide bridge between Cys 1 and Cys 6 is shown.

Several other areas of the CNS have also been described to possess nonmagnocellular OT-secreting neurons, like the hypothalamic nucleus, thalamic nuclei, hippocampus, amygdala, olfactory bulbs and others, suggesting a role for OT as neurotransmitter in these areas. Within the CNS, AVP regulates neuronal communication, feeding, thermoregulation and behavioural functions such as memory, social and sexual processes, anxiety and depression. Both neuropeptides are also an integral part of the mammalian emotional circuitry and are associated with the emergence of social bonding, parental care, stress regulation, social communication and emotional reactivity.

In addition, several other organs, like the heart, ovary, amnion, chorion, decidua, testis, epididymis and prostate, have been reported to synthesize OT, suggesting a paracrine role for this hormone in these tissues. Ectopic AVP production by lung cancer cells or other neoplasms has been described in humans, leading to ►the syndrome of inappropriate antidiuretic hormone secretion.

The stimuli for AVP secretion are plasma hyperosmolality (high sodium), hypovolaemia and hypotension. In addition, potent stimuli are nausea and vomiting, a less potent stimulus is hypoglycaemia. Several drugs induce AVP secretion, either directly, indirectly or by unknown mechanisms, like vincristine, cyclophosphamide, tricyclic antidepressants, apomorphine, nicotine, high doses of morphine and lithium. Lithium however also inhibits the renal effects of AVP. The secretion of AVP is suppressed by ►atrial natriuretic peptide (ANP), ethanol, opioids (particularly dynorphin), low doses of morphine, phenytoin, dopaminergic antagonists (fluphenazine, haloperidol, promethazine) and carbamazepine, which also has a renal antidiuretic action.

OT secretion is triggered by the stimulation of the nipples, resulting in the milk ejection reflex, and by the distension of cervix and vagina during labour. OT release is also stimulated by plasma hyperosmolality (high sodium) and hypervolaemia, suggesting a regulatory role in natriuresis and blood volume for this hormone. Secretion also occurs during sexual arousal and ejaculation. Higher OT levels are associated with socially pleasant sensory experiences, such as touch and smell. OT secretion is suppressed by alcohol and opioids.

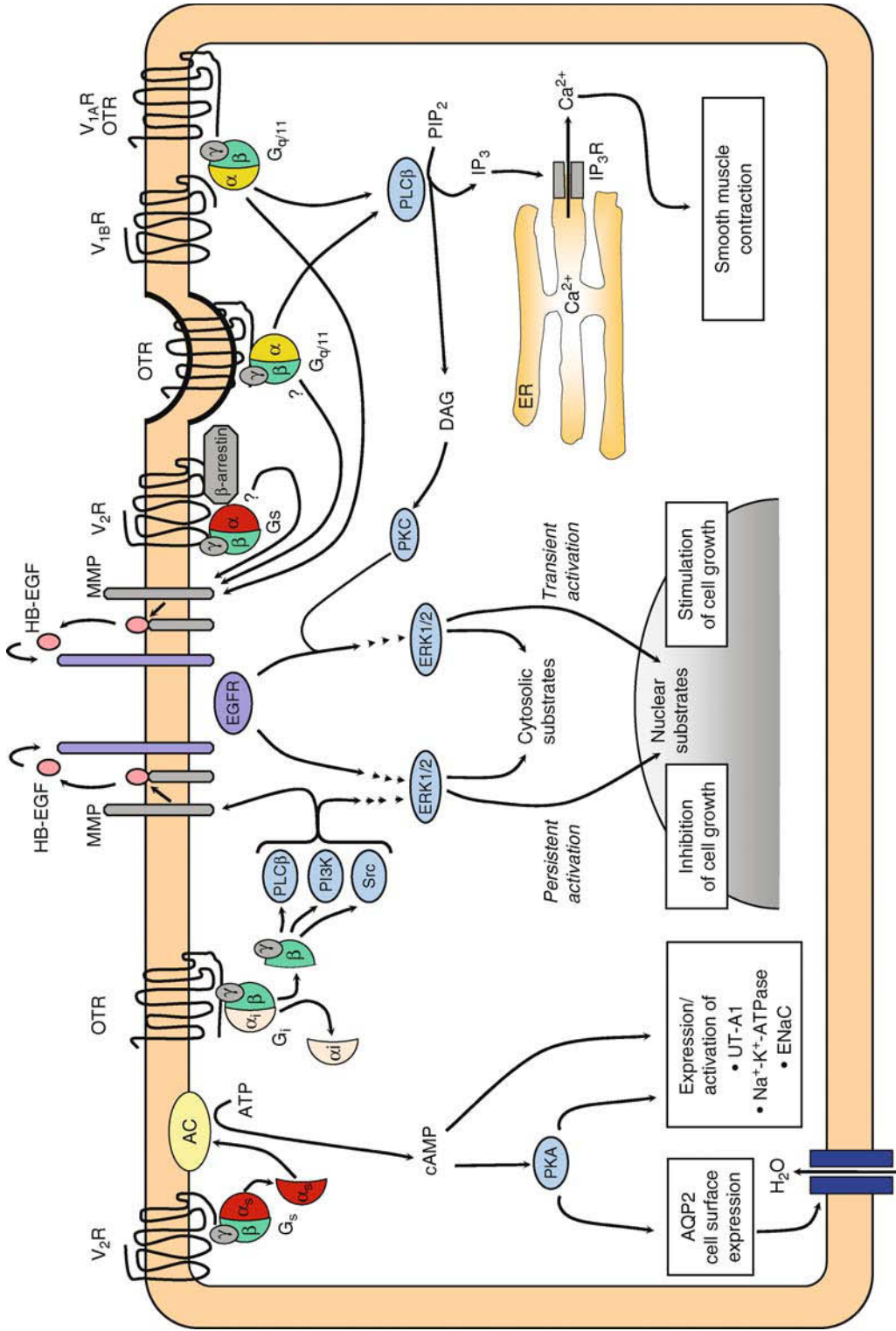
AVP and OT elicit their physiological and pharmacological roles through cell surface receptors. The canonical receptors for AVP and OT form a subfamily within the ►G protein-coupled receptor (GPCR) family. Three different subtypes of AVP receptors are known ($V_{1A}R$, $V_{1B}R$, V_2R). A fourth receptor that binds AVP is the vasopressin-activated calcium-mobilizing receptor (VCAM-1/cul-5) that belongs to the cullin gene family. So far, there is only one type of OTR known.

AVP Receptors

The $V_{1A}R$ and $V_{1B}R$ selectively couple to ►heterotrimeric GTP-binding proteins (G proteins) of the $G_{q/11}$ family, which mediate the activation of distinct isoforms of phospholipase C_β (PLC_β) and extracellular-regulated kinase (ERK1/2) (Fig. 2). Both receptors have been reported to activate also PLD and PLA_2 . Activated V_{1R} s catalyse the dissociation of the heterotrimeric G proteins into the $G_{\alpha q/11}$ subunit and the $G_{\beta\gamma}$ heterodimer. The activated $G_{\alpha q/11}$ subunit stimulates PLC_β , which catalyses the hydrolysis of phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 diffuses through the cytosol and binds to the IP_3 receptor (IP_3R), an intracellular ion channel that mediates the release of calcium from the endoplasmic reticulum (ER). DAG activates protein-kinase C (PKC) and the ERK1/2 pathway, which leads to the activation of Elk and c-Myc inducing cell growth and differentiation. The V_2R activates the G protein G_s , leading to the activation of adenylyl cyclase (AC) and consequently to an increase of cytosolic cyclic adenosine-monophosphate (cAMP). Stimulation of V_2R s also leads to β -arrestin recruitment to the plasma membrane and to the activation of ERK1/2; a G_s -dependent and -independent pathway has been proposed, involving PKA, Raf-1, c-Src, receptor tyrosine kinase transactivation (MMP, HB-EGF, EGRF), MEK1/2 (Fig. 2). The precise function of V_2R -regulated ERK1/2 activity in vivo is still unknown, but it might play a role in abnormal cell growth or differentiation (polycystic kidney disease, tumor growth).

The $V_{1A}R$ mediates the extrarenal actions of AVP such as vasoconstriction, platelet aggregation and hepatic glycogenolysis by increasing cytosolic calcium concentrations. The $V_{1A}R$ is expressed in the liver (hepatocytes surrounding central veins), vascular and gastrointestinal smooth muscle, bladder, myometrium, platelets, the renal medulla, iris and throughout the brain. It is believed that $V_{1A}R$ function is essential for an efficient response to hypovolemic stress conditions, e.g. hemorrhagic shock, where AVP is secreted copiously. The $V_{1A}R$ in the brain is proposed to mediate the effects of AVP on memory, learning, antipyresis, brain development, selective aggression, partner preferences in rodents, cerebrospinal fluid production and analgesia. The $V_{1B}R$ is mainly expressed in humans in the anterior pituitary, adrenal medulla and kidneys, and believed to be present in the CNS. The activation of $V_{1B}R$ potentiates the response to CRF in the anterior pituitary lobe that leads to ACTH secretion into the systemic circulation and is involved in the genesis of anxiety and depression.

The V_2R is expressed in collecting duct cells; in rodents it is also expressed in cells of the thick ascending limb of Henle's loop (TAL). V_2R -induced G_s -mediated activation of AC, the subsequent increase



Vasopressin/Oxytocin. Figure 2 Schematic representation of the major signal transduction pathways of AVP and OT receptors. The signal transduction pathway and effects of OTRs do vary depending on the receptor's plasma membrane localization, e.g. in caveolae. Pharmacological intervention is possible by the use of selective agonists (AVP or OT analogues) or receptor antagonists. EGFR, epidermal growth factor receptor; HB-EGF, heparin-binding EGF-like growth factor; MMP, matrix metalloproteinase.

in cytosolic cAMP and the stimulation of PKA lead to the final step of the antidiuretic actions of AVP: the exocytic fusion of intracellular vesicles bearing the selective water channel ►aquaporin-2 (AQP2) with the plasma membrane of principal collecting duct cells (short-term regulation). The water permeability of the cells increases dramatically (10–20 fold), allowing not only the entry of water via the apical membrane but also the exit across the basolateral membrane through constitutively expressed water channels (AQP3 and AQP4). Due to the osmotic gradient of the inner medulla, water accumulates in the interstitium and is taken up by the circulation. AVP withdrawal is associated with endocytic retrieval of ►AQP2 back into intracellular vesicles. Activation of V₂R also stimulates the synthesis of ►AQP2 (long-term regulation) in principal cells; it also regulates the expression of the ►urea transporter A1 (►UT-A1) and activates it via PKA, thereby increasing urea absorption. In addition, it regulates the long-term expression and activation of the Na⁺-K⁺-2Cl⁻ symporter in the TAL. It enhances Na⁺ transport in the collecting duct by an increased expression of subunits of the epithelial sodium channel (ENaC). These effects contribute to an increase in medullary osmolality and thereby to an enhancement of renal water conservation. There is strong evidence for extrarenal V₂R expression in lungs, heart and skeletal muscle, inner ear, small-cell lung cancer, colon epithelial and endothelial cells. The administration of a V₂R agonist (desmopressin) to patients suffering from X-linked congenital nephrogenic DI (*AVPR2* gene defect) does not result in the usual increase of factor VIII, von Willebrand factor and tissue plasminogen activator in the blood. The therapeutic use of desmopressin for the treatment of coagulation disorders, like haemophilia A and von Willebrand's disease (type I), relies on the existence of extrarenal V₂R.

The VACM-1 receptor is a membrane-associated protein with a single putative transmembrane domain that binds selectively AVP ($K_D = 2$ nM), but cannot discriminate between V₁R and V₂R analogues. It is expressed in endothelial and medullary collecting duct cells and upon stimulation by AVP. It induces a mobilization of cytosolic-free Ca²⁺, decreases cAMP production and inhibits cellular growth via MAPK phosphorylation and p53 expression. The mechanism of action and physiological functions of this new receptor are not well understood, but it seems to participate in the regulation of AVP induced signal transduction pathways or of a yet unidentified peptide.

Congenital Nephrogenic Diabetes Insipidus (X-linked, Autosomal)

Congenital nephrogenic diabetes insipidus (NDI) is a rare disease, characterized by an inability to concentrate urine despite normal or elevated AVP plasma

concentrations. The syndrome is characterized by polyuria and polydipsia. If not treated, episodes of dehydration can result in mental retardation in the newborn. Other symptoms include vomiting, anorexia, failure to thrive and constipation. The more common form is X-linked NDI, caused by different mutations in the *AVPR2* gene. The excessive water loss through the kidney is due to an improper V₂R function. Mutations of the V₂R can lead to: (i) an altered affinity for AVP (►K_D increased) or (ii) an altered coupling to the G_s protein (►EC₅₀ increased), (iii) an altered mRNA synthesis or stability and (iv) the most common form, an altered transport to the cell surface (intracellular retained receptors). The autosomal NDI form, caused by mutations in the *AQP2* gene, is extremely rare [3]. In contrast to patients suffering from the X-linked NDI form, patients with the autosomal form show the typical coagulation response to desmopressin.

OTR

There is only one OTR known so far. Nevertheless, ►posttranslational modifications, localization in special plasma membrane compartments (lipid rafts or caveolae) and interactions with downstream signal transduction components modify OTR signalling. OTRs activate PLC β and increase cytosolic Ca²⁺ (Fig. 2). These effects are transduced by the activation of the G protein G_{q/11}. The increase in cytosolic calcium concentrations is the main trigger for smooth muscle contraction. OTR activation also leads to activation of ERK1/2 in myometrial and endothelial cells (proliferation), an effect that involves $\beta\gamma$ release from G_{q/11} and transactivation of the EGFR. In decidua activation of OTR induces PGF_{2 α} release, which enhances uterine contractions, cervical ripening and luteolysis. This effect is G_i- and G_{q/11}-dependent and involves a Ca²⁺-mediated translocation of PLA₂ from the cytoplasm to cell membranes, in addition to the activation of PLA₂ by MAPK. When only a minor fraction of OTRs is located in lipid rafts (caveolae), they inhibit cell proliferation (via G_i, PLC, c-Src, PI3K, EGFR transactivation and persistent ERK1/2 activation), but when targeted to lipid rafts they have a strong mitogenic effect (via G_{q/11}, $\beta\gamma$ release, EGFR transactivation and transient ERK1/2 activation) (Fig. 2).

OTRs are mainly expressed in myoepithelial cells of the galactiferous channels and the myometrium. The OTRs in vascular endothelial cells, renal epithelial cells (macula densa, proximal tubule) and cardiomyocytes induce the production of NO (vasodilation), natriuresis and release of ANP, respectively. The endometrium, ovary, amnion, testis, epididymis, prostate and thymus also express the OTR supporting a paracrine role of this peptide. Osteoblasts, osteoclasts, pancreatic islets cells, adipocytes, and several types of cancer cells also express OTRs. More over, expression of the OTR

has also been described in the brain (hypothalamus, nucleus accumbens, neurons and astrocytes), with patterns differing in sex, age and species, which may be related to differences in partnership, sexual, maternal and social behaviour.

There are several hints indicating a strong interaction between OTR expression and sexual hormones. During pregnancy, high plasma progesterone levels promote uterine relaxation and inhibit the function of the OTR by both genomic and nongenomic mechanisms. The myometrial OTRs are abundantly expressed in the last days of pregnancy, after estrogen secretion. Possibly progesterone and estrogens act in opposing manner on the function, expression, and/or regulation of OTRs. However, despite the striking dependence of the OT system on gonadal hormones, the human *OTR* gene does not contain a classical steroid responsive element and nongenomic mechanisms are not well characterized. Mechanical stretch is an important local stimulus for myometrial OTR expression at term. Although OT plasma concentrations remain relatively constant until labour itself, uterine sensitivity to OT is markedly increased around onset of labour. This is associated with a strong increase in the density of myometrial OTRs, reaching a peak during early labour (200 times higher than in the nonpregnant state). Thus, at onset of labour, OT can stimulate uterine contractions at plasma concentrations that are ineffective in the nonpregnant state. After parturition, the concentrations of uterine OTR rapidly decline. Downregulation of OTR may be necessary to avoid unwanted contractile responses during lactation, when OT plasma levels are raised [1].

Although the OT system may be regarded as a key regulator of labour, in OT-deficient mice, parturition remains unaffected. Moreover, OTR knockout mice do deliver in a normal fashion, but the offspring die during the very first days of life, due to starving, as the milk ejection reflex is absent in these animals. These experiments show that the OT system is not essential for labour or reproductive behaviour (at least in mice), but for the milk ejection reflex, which is fundamental for litter survival.

OTR is also expressed in male reproductive tissues, like testis, epididymis and in the prostate. OT increases the resting tone of prostatic tissue from guinea pig, rat, dog and human. The activation of these receptors could lead to the contraction of the prostate and the resulting expulsion of prostatic secretions during ejaculation.

Drugs

AVP Receptors

Agonists

AVP is mainly used in the replacement therapy in ►congenital diabetes insipidus (CDI), but has only a short effect of 4–6 h. It is also used in the therapy of gastrointestinal haemorrhage, as a local vasoconstrictor,

as a vasopressor during cardiac arrest and in the treatment of adult shock-refractory ventricular fibrillation. The major disadvantages of AVP in ►CDI therapy are its nonselective effects ($V_{1A}/V_{1B}R$ stimulation), short duration and contraction of the uterus and gastrointestinal tract. Otherwise, AVP is used to treat postoperative ileus and abdominal distension and to eliminate gas before abdominal radiographs.

The synthetic AVP analogue 1-deamino,8-D-arginine vasopressin (dDAVP) or desmopressin, is a mixed $V_{1B}/V_{2}R$ agonist in human. Its antidiuretic effect is longer than AVP and it does not cause vasoconstriction and is better tolerated than AVP. Desmopressin is used in the treatment of ►CDI, by intranasal spray application. It is also used in the therapy of nocturnal enuresis in some countries. Another important clinical application of desmopressin is the treatment of haemophilia A (factor VIII) and von Willebrand's disease (type I). Lysipressin (8-lysine-vasopressin) is also available for intranasal application. Terlipressin (triglycyl-lysine-vasopressin) a synthetic analogue of lysipressin is released in a slow and sustained manner permitting intermittent administrations. Side effects of desmopressin include vasodilatation that causes facial flushing, headache, nausea and occasional tingling. The major complication of AVP, lysipressin, desmopressin and terlipressin is water intoxication with hyponatraemia (iatrogenic syndrome of inappropriate antidiuretic hormone secretion).

OPC-51803 is a highly selective $V_{2}R$ agonist and may prove useful for the treatment of ►CDI, urinary incontinence, enuresis and pollakiuria. It has a much higher bioavailability after oral application than desmopressin.

Antagonists

Several nonpeptidic, orally active vasopressin receptor antagonists have been developed. The dual $V_{1A}/V_{2}R$ antagonist conivaptan is used in the treatment of hyponatraemia and could also become useful for diseases such as congestive heart failure, in which increased peripheral resistance and dilutional hyponatraemia both are present [4]. Side effects of conivaptan include headache, injection site reactions, vomiting, diarrhoea, constipation and thirst.

Relcovaptan (SR-49059) is a selective, orally active $V_{1A}R$ antagonist that prevents pain of primary dysmenorrhea and inhibits preterm labour and could be useful in the treatment of Raynaud's phenomenon. The selective $V_{1B}R$ antagonist SSR149415 showed beneficial effects in the treatment of depression and anxiety in several animal models.

Several selective $V_{2}R$ antagonists have also been developed (tolvaptan, lixivaptan, OPC-31260, Satavaptan, RWJ-351647). These substances, together with conivaptan, are also known as "►aquaretic agents".

Their main applications are the treatment of water disorders caused by congestive heart failure, liver cirrhosis, nephrotic syndrome, ocular hypertension and syndrome of inappropriate antidiuretic hormone secretion. Tolvaptan (OPC-41061) was successfully applied for the treatment of euvoemic or hypervolaemic hyponatraemia in two phase 3 studies (SALT-1 & SALT-2). It could also be used for polycystic kidney disease, since it antagonizes effectively the proliferative responses to the ►cAMP - B-Raf – Ras - ERK1/2 pathway. Some antagonists (conivaptan, relcovaptan, Satavaptan) are also known to have specific “chaperonal” activity, helping intracellularly retained NDI-causing V₂R mutants to fold to a native conformation and rescue their functional activity (►pharmacological chaperone). This is a new promising therapeutic approach for the treatment of congenital NDI and several other hereditary diseases that result from protein misfolding and intracellular transport.

OTR Agonists

OT is mainly used intravenously for labour induction without major side effects. It is also used to reduce postpartum bleeding and to facilitate milk ejection in mastitic cows. Carbetocin, a long-acting OT analogue with modifications in the terminal cysteine residue and in the disulphide bridge, is used to control bleeding after delivery. Orally available nonpeptide compounds are being developed for the treatment of various conditions including male erectile dysfunction, labour promotion and control of postpartum bleeding and milk letdown.

Antagonists

Atosiban is a combined OTR and V_{1A}R antagonist peptide that is successfully used intravenously as a tocolytic in preterm labour. This antagonist was shown to inhibit uterine contractions in women with threatened and established preterm labour, with a favourable side effect profile, when compared to ritodrine (β₂-sympathomimetic). A potential use of atosiban is in benign prostate hyperplasia, as it is known that OTRs play an important role in prostate contraction. Interestingly, atosiban acts also as a biased or protean agonist. It acts as an antagonist over the G_{q/11}-mediated pathway (uterus contraction) and as an agonist over the G_i-mediated pathway (inhibition of cell growth) of the OTR. This particular phenomenon is termed “agonist directed trafficking of receptor stimulus”. Substance-P analogues (SP-G/-D) are an example of protean agonists over V_{1A}R.

Barusiban is a long-acting, selective antagonist of OTR that is in development for preterm labour. It has a 300-fold higher affinity for the OTR than for the V_{1A}R and hence more selective action than atosiban on myometrial tissues. The duration of action may be longer than that of atosiban, providing the convenience

of less frequent administration in the clinic. Other nonpeptide OTR antagonists are under development [5].

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Vasorelaxant

►Vasodilator

Vav

Vav proteins are guanine nucleotide exchange factors (GEF) for monomeric GTPases. The Vav proteins belong to the DBL family of Rho GEFs and have an important role in regulating early events in receptor signalling.

- Phospholipid Kinases
- Small GTPases

VEGF

►Vascular Endothelial Growth Factor

Venus Flytrap Module

Synonyms VFT Module

Definition

The VFT module is a protein module first characterized in bacteria, where it is involved in the transport through the periplasm of small molecules like amino acids, ions, peptides and sugars. Since then, a similar protein module has been shown to regulate the activity of operons (like the amidase operon that controls the expression of the amidase enzyme) and to be in the guanylyl cyclase and metabotropic glutamate receptors, for which it constitutes the ligand binding domain. This module is composed of two lobes interconnected by two or three linkers. In most cases, the two lobes are separated by a wide cleft in which the ligand binds. Binding of the ligand stabilizes a closed form of the protein, so that the ligand is trapped like an insect trapped between the two lobes of the leaves of the carnivorous plant “Venus Flytrap”.

- ▶ G-protein-coupled Receptors
- ▶ Ca^{2+} -sensing Receptor

Very Long-chain Acyl-CoA Synthetase

- ▶ Fatty Acid Transporters

Very Low-density Lipoprotein

Synonyms

VLDL

Definition

Lipoprotein fraction containing triglycerides and to a lesser degree cholesterol. VLDL is produced by the liver. The main structural protein connected to this lipoprotein class is apolipoprotein B.

- ▶ Lipid Transfer Proteins
- ▶ Lipoprotein Metabolism

Vesicle

Vesicles are transport containers that are formed upon recruitment of coat proteins from a donor membrane that fuse with an acceptor membrane.

- ▶ Intracellular Transport
- ▶ Exocytosis

Vesicular Acetylcholine Transporter

VACHT.

- ▶ Vesicular Transporters

Vesicular Transporters

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Synonyms

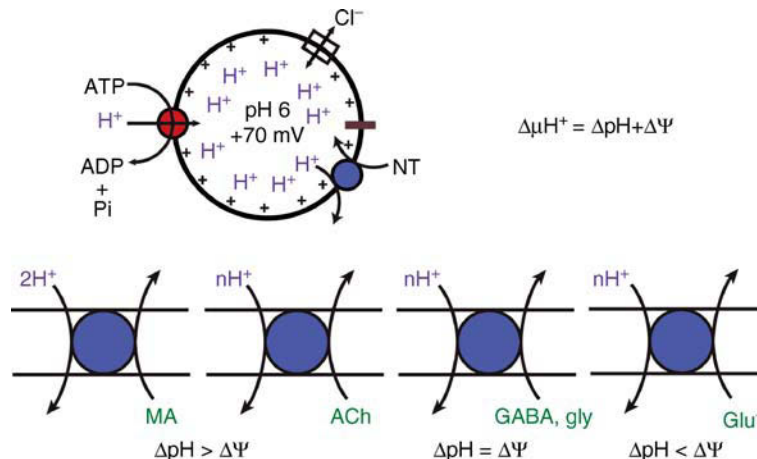
Synaptic vesicle neurotransmitter transporters; VMAT1; VMAT2; VGAT/VIAAT; VGLUT1; VGLUT2; VGLUT3; VACHT

Definition

The exocytotic release of neurotransmitters from synaptic vesicles underlies most information processing by the brain. Since classical neurotransmitters including monoamines, acetylcholine, GABA, and glutamate are synthesized in the cytoplasm, a mechanism is required for their accumulation in synaptic vesicles. Vesicular transporters are multitransmembrane domain proteins that mediate this process by coupling the movement of neurotransmitters to the proton electrochemical gradient across the vesicle membrane.

Basic Characteristics

Synaptic vesicles isolated from brain exhibit four distinct vesicular neurotransmitter transport activities: one for monoamines, a second for acetylcholine, a third for the inhibitory neurotransmitters GABA and glycine, and a fourth for glutamate [1]. Unlike Na^+ -dependent plasma membrane transporters, the vesicular activities couple to a proton electrochemical gradient ($\Delta\mu_{\text{H}^+}$) across the vesicle membrane generated by the vacuolar H^+ -ATPase (▶ vacuolar type proton translocating ATPase). Although all of the vesicular transport systems rely on $\Delta\mu_{\text{H}^+}$, the relative dependence on the chemical and electrical components varies (Fig. 1). The



Vesicular Transporters. Figure 1 Stoichiometry and bioenergetic dependence of the vesicular neurotransmitter transporters. The proton electrochemical gradient ($\Delta\mu_{H^+}$) that drives neurotransmitter transport into secretory vesicles is generated by the vacuolar-type proton translocating ATPase and consists of a 1–2 unit pH gradient (ΔpH) and a transmembrane potential ($\Delta\Psi$) $\sim +70$ mV (inside positive). The different families of transport proteins exhibit different dependences on these two components of the gradient, with VMATs and VACHT most dependent on ΔpH , VGLUTs on $\Delta\Psi$, and VGAT dependent on both. These differences in dependence on $\Delta\mu_{H^+}$ reflect differences in the stoichiometry of transport. Abbreviations: VMATs, vesicular monoamine transporters; VACHT, vesicular acetylcholine transporter; VGAT, vesicular GABA transporter.

vesicular monoamine and acetylcholine transport systems depend primarily on the chemical component of this gradient (ΔpH), whereas transport of GABA relies on both ΔpH and the electrical component of the gradient ($\Delta\Psi$), and glutamate transport almost exclusively on $\Delta\Psi$. These bioenergetic differences appear to reflect differences in protein structure.

Vesicular Monoamine Transport

The vesicular monoamine transporters (VMATs) were identified in a screen for genes that confer resistance to the parkinsonian neurotoxin MPP⁺ [2]. The resistance apparently results from sequestration of the toxin inside vesicles, away from its primary site of action in mitochondria. In addition to recognizing MPP⁺, the transporters mediate the uptake of dopamine, serotonin, epinephrine, and norepinephrine by neurons and endocrine cells. Structurally, the VMATs show no relationship to plasma membrane monoamine transporters.

VMAT1 is expressed in the adrenal medulla, by small intensely fluorescent cells in sympathetic ganglia, and by other nonneural cells that release monoamines. In contrast, VMAT2 is expressed by neuronal populations in the nervous system. The substrate specificity for the two isoforms is similar, but VMAT2 has a somewhat higher apparent affinity for all monoamines than VMAT1. In addition, only VMAT2 appears able to transport histamine, consistent with its expression by mast cells.

Transport by the VMATs involves the exchange of two luminal protons for one cytoplasmic, apparently protonated molecule of transmitter, predicting accumulation of transmitter inside vesicles 10^4 – 10^5 the

concentrations in cytoplasm [3]. Using reserpine binding (see below), a number of residues appear to be required for substrate recognition; reserpine still binds to these mutants, but cannot be displaced by substrates. In addition, $\Delta\mu_{H^+}$ accelerates reserpine binding to wild-type VMAT2, suggesting that H⁺ efflux reorients the substrate recognition site to the cytoplasmic face of the membrane. The substrate recognition mutants do not affect this stimulation by $\Delta\mu_{H^+}$. However, substitution of an aspartate in predicted transmembrane domain (TMD) 10 of the VMATs by a neutral residue abolishes reserpine binding even in the presence of $\Delta\mu_{H^+}$, and replacement by glutamate shifts the pH sensitivity of transport, suggesting that this residue contributes to H⁺ translocation. In terms of structure, a lysine in TMD2 also appears to form a charge pair with an aspartate in TMD11.

Vesicular Acetylcholine Transport

Closely related to the VMATs, the vesamicol-sensitive vesicular acetylcholine transporter (VACHT) was identified in a screen for mutants resistant to inhibition of acetylcholinesterase in *C. elegans* [1]. Like VMATs, VACHT recognizes a cationic substrate and depends primarily on ΔpH [2]. In contrast to VMATs, with substrate affinities in the low or submicromolar, the mammalian VACHT exhibits an apparent affinity around 1 mM. The higher affinity of VMATs may reflect the need to lower cytoplasmic levels of potentially toxic monoamine transmitters. However, VACHT has been postulated to have a similar stoichiometry for 2 H⁺, but to exhibit as well an H⁺ leak not present with the VMATs. VACHT is expressed

in cholinergic neurons in the central, peripheral, and autonomic nervous systems.

Vesicular GABA Transport

Genetic and behavioral studies in the nematode *C. elegans* led to identification of the vesicular GABA transporter VGAT/vesicular inhibitory amino acid transporter (VIAAT) [4]. As predicted from studies with purified synaptic vesicles, VGAT mediates uptake of GABA with an apparent affinity in the low millimolar range and can be driven by ΔpH or $\Delta\psi$. VGAT also recognizes glycine as a substrate, suggesting that these two inhibitory neurotransmitters can be stored in and released from the same vesicle. VGAT is expressed in GABAergic, glycinergic neurons as well as in the pancreas.

VGAT shows no sequence similarity to VMATs or VACHT. Rather, it belongs to a large family of transporters that includes H^+ -coupled amino acid permeases in *Arabidopsis thaliana* and several mammalian proteins. One of the related mammalian transport proteins corresponds to classical amino acid transport system N, and others correspond to classical system A, which is responsible for much of the active amino acid uptake by mammalian cells. These glutamine transporters appear to mediate aspects of the glutamine–glutamate cycle involved in nitrogen metabolism by the liver and in the regeneration of glutamate from glutamine required for excitatory neurotransmission. The system N transporters SN1/SNAT3 and SNAT5 mediate glutamine efflux readily under physiological conditions and are expressed by astrocytes. In contrast, system A transporters SA1/SNAT2 and SA2/SNAT1 primarily mediate glutamine uptake, and the expression of SA1 and SA2 by neurons positions these transporters for the uptake of glutamine released from astrocytes by SN1. The principal difference between system N and A transporters appears to be the translocation of H^+ ; the system N transporters mediate the exchange of H^+ for Na^+ and an amino acid whereas the system A transporters mediate only Na^+ cotransport.

Vesicular Glutamate Transport

Glutamate is the principal excitatory neurotransmitter in the mammalian central nervous system, yet the vesicular glutamate transporter eluded identification until recently [5]. Initially isolated in a screen for genes upregulated by subtoxic doses of the excitotoxin N-methyl-D-aspartate, the brain-specific Na^+ -dependent phosphate transporter (BNPI) resembled a class of ►inorganic phosphate transporters and conferred Na^+ -dependent phosphate transport in *Xenopus* oocytes. Although suggested to participate in the regeneration of ATP at nerve terminals, BNPI expression is restricted to excitatory neurons, suggesting a more specific role in glutamate release. Indeed, the enzyme glutaminase that

produces glutamate for release as an excitatory transmitter requires inorganic phosphate for activity. Further, studies in *C. elegans* indicated a specific role for the BNPI orthologue EAT-4 in glutamate release. Recent work has suggested that other type I phosphate transporters related to BNPI mediate the transport of organic anions with higher affinity than phosphate. Together with the determination that BNPI localizes to synaptic vesicles, this suggested an alternative role for the protein in vesicular glutamate transport.

Heterologous expression of BNPI in a variety of cell systems indeed confers vesicular glutamate uptake with all of the properties demonstrated using synaptic vesicles from brain. In particular, glutamate uptake by BNPI depends primarily on $\Delta\psi$ rather than ΔpH , does not recognize aspartate, has an apparent affinity in the low millimolar, and shows a biphasic dependence on chloride with an optimum at 2–10 mM. BNPI has thus been renamed VGLUT1. In addition, vesicular glutamate transport by VGLUT1 leads to vesicle acidification, as previously demonstrated using native synaptic vesicle preparations. Presumably, a reduction in electrical potential caused by influx of the anionic glutamate secondarily activates the H^+ -ATPase. However, VGLUT1 and other related proteins also appear to exhibit a substantial chloride conductance that is blocked by substrate.

Although the expression of VGLUT1 is limited to a subset of glutamatergic neurons in the brain, a highly homologous protein, also initially characterized as a phosphate transporter and named differentiation-associated Na^+ -dependent phosphate transporter (DNP1), has a complementary pattern of expression. DNP1 also localizes to synaptic vesicles, mediates the vesicular uptake of glutamate and has been renamed VGLUT2. Although VGLUT2 exhibits transport activity very similar to VGLUT1, VGLUT2 appears to be expressed by a subset of glutamate neurons with a higher probability of release, and differences in subcellular location suggest differences in trafficking. In particular, the presence of two polyproline domains in the C-terminal cytoplasmic tail of VGLUT1 mediate interactions with endophilin, an SH3 domain containing protein that has been shown to be involved in synaptic vesicle recycling. A third vesicular glutamate transporter, VGLUT3 has also been identified. VGLUT3 is expressed in neurons not classically considered glutamatergic. Immunohistochemical and in situ studies indicate VGLUT3 is expressed in GABAergic, serotonergic, dopaminergic, and cholinergic neurons as well as astrocytes.

Vesicular Storage of Zinc, ATP, and Neuropeptides

Synaptic vesicles mediate the release of small molecules other than classical neurotransmitters and neuropeptides. Of these, zinc and ATP are the best characterized. NMDA and GABA receptors contain binding sites for zinc, and zinc exerts a direct effect on

excitatory and inhibitory neurotransmission. ATP activates both ionotropic and G protein-coupled receptors. As with the classical neurotransmitters, the exocytotic release of these compounds requires transport into synaptic vesicles.

ZnT3 has been implicated in zinc uptake by synaptic vesicles. ZnT3 belongs to a family of zinc transporters and localizes to synaptic vesicles. Further, mice deficient in ZnT3 show a loss of zinc staining from hippocampal neurons and expression of ZnT3 in PC12 cells increases vesicular zinc staining when coexpressed with the vesicular glutamate transporter VGLUT1. ZnT3 targeting to synaptic vesicles has also been shown to depend on the adaptor protein AP3. In *mocha* mice deficient in AP3, synaptic vesicles accumulate normally, but no longer contain ZnT3 or zinc, further supporting a role for ZnT3 in synaptic vesicle zinc storage.

Chromaffin granules, platelet dense core vesicles, and synaptic vesicles accumulate ATP. ATP uptake has been demonstrated using chromaffin granules and synaptic vesicles and the process appears to depend on $\Delta\mu_{H^+}$. It has generally been assumed that ATP is costored only with monoamines and acetylcholine, as an anion to balance to cationic charge of those transmitters. However, the extent of ATP storage and release by different neuronal populations remains unknown, and the proteins responsible for ATP uptake by secretory vesicles have not been identified.

Unlike classical neurotransmitters, neuropeptides enter the lumen of the secretory pathway in the endoplasmic reticulum, through cotranslational translocation. They then sort to large dense core vesicles (LDCVs) in the trans-Golgi network (TGN) and undergo processing to form the biologically active species. LDCV exocytosis exhibits a different dependence from synaptic vesicles on stimulation and calcium concentration. After release, neuropeptides undergo degradation; they are generally not repackaged, although certain luminal contents of dense core vesicles can remain tethered to the plasma membrane.

Regulation of Vesicular Transporters

Regulation of transmitter release may involve changes in the probability of release, or in the amount of transmitter per synaptic vesicle. A variety of mechanisms are known to regulate the probability of vesicle release. Similarly, changes in the amount of transmitter per vesicle, or quantal size, occur in several systems, including the neuromuscular junction. The mechanisms by which quantal size changes, however, remain less well characterized. Nonetheless, modulation of vesicular transport can clearly influence quantal size. VMAT2 knockout mice die shortly after birth, but heterozygous animals also exhibit substantial reductions in monoamine

release, and alterations in behavior relative to wild-type litter mates [4]. Conversely, overexpression of either VMAT2 or VACHT can increase quantal size.

Regulation of transporter expression may occur at the transcriptional level. Both VGLUTs were identified as genes upregulated by specific stimuli; VGLUT1 in response to an excitotoxin and VGLUT2 to the growth factor activin. The organization of the VACHT gene within an exon of the biosynthetic enzyme choline acetyl transferase further indicates a remarkable level of transcriptional coordination. In addition to transcriptional regulation of the VGLUTs, VMATs appear to be regulated by G proteins. Activation of the G protein $G\alpha_{O2}$ downregulates their activity independent of $\Delta\mu_{H^+}$, but the mechanism remains unclear.

The major mechanisms for regulation of vesicular neurotransmitter transport appear to involve changes in membrane trafficking. VMATs undergo phosphorylation by casein kinase and this posttranslational modification influences their retrieval from maturing LDCVs. In addition, phosphorylation of VACHT upstream from a dileucine-like motif influences sorting into LDCVs at the level of the TGN. Since sorting to LDCVs versus synaptic vesicles will determine the site and mode of transmitter release, the regulation of transporter trafficking has great potential to influence signaling. In particular, it is well known that midbrain neurons release dopamine from their cell bodies and dendrites as well as from their terminals, and the trafficking of VMAT2 presumably contributes to these two very different modes of release. Interactions between VGLUT1 and endophilin have also been implicated in the regulation of synaptic vesicle recycling.

Drugs

VMATs are irreversibly inhibited by the potent antihypertensive drug reserpine. The depressive effects of reserpine helped to formulate the original monoamine hypothesis of affective disorders. Reserpine also appears to interact with the transporters near the site of substrate recognition. Tetrabenazine, which is used in treatment of movement disorders, inhibits VMAT2 much more potently than VMAT1, consistent with the less hypotensive action of this agent.

VMATs are not inhibited by drugs such as cocaine, tricyclic antidepressants and selective serotonin reuptake inhibitors that affect plasma membrane monoamine transport. Amphetamines have relatively selective effects on monoaminergic cells due to selective uptake by plasma membrane monoamine transporters, but their effect appears to be mediated by their ability as weak bases to reduce ΔpH , the driving force for vesicular monoamine transport that leads to efflux of the vesicular contents into the cytoplasm.

The vesicular acetylcholine transport can be inhibited by vesamicol and several related compounds. Vesamicol competitively inhibits transport by binding to a cytoplasmic domain on VACHT with a K_d of ~ 5 nM. Vesamicol binding can be used to estimate transporter number, but neither vesamicol nor its analogues are currently used clinically.

Vesicular GABA transport can be competitively inhibited by amino acids including glycine and β -alanine. Transport can also be competitively inhibited by γ -vinyl GABA, a derivative of GABA. γ -Vinyl GABA has been used in the clinical treatment of epilepsy and is known to inhibit GABA transaminase, an enzyme that metabolizes GABA. The mode of action for γ -vinyl GABA as an antiepileptic drug is thought to be through its effects on GABA transaminase, but inhibition of vesicular GABA transport could have an effect by increasing the nonvesicular release of GABA.

Several compounds that inhibit vesicular glutamate transport have been identified: These include the dyes Evans Blue and Rose Bengal. In addition, the stilbene derivative 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), a compound commonly used as a specific inhibitor of anion channels, inhibits vesicular glutamate transport. Most known inhibitors have limited use as they are membrane impermeant, with the exception of Rose Bengal.

In addition to direct inhibition of the vesicular transport protein, storage of neurotransmitters can be reduced by dissipation of the proton electrochemical gradient. Bafilomycin (a specific inhibitor of the vacuolar H^+ -ATPase), as well as the proton ionophores carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and carbonylcyanide *p*-(trifluoromethoxy) phenylhydrazone (FCCP) are used experimentally to reduce the vesicular storage of neurotransmitters. Weak bases including amphetamines and ammonium chloride are used to selectively reduce ΔpH .

- [Neurotransmitter Transporters](#)
- [Organic Cation Transporters](#)

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VFT Domain

- [Venus Flytrap Module](#)

VGAT/VIAAT

Vesicular GABA transporter; vesicular inhibitory amino acid transport.

- [Vesicular Transporters](#)

VGLUT

VGLUT is short for vesicular glutamate transporter.

- [Vesicular Transporters](#)

Vinca Alkaloids

Vinca alkaloids are derived from the Madagascar periwinkle plant, *Catharanthus roseus*. The main alkaloids are vincristine, vinblastine and vindesine. Vinca alkaloids are cell-cycle-specific agents and block cells in mitosis. This cellular activity is due to their ability to bind specifically to tubulin and to block the ability of the protein to polymerize into microtubules. This prevents spindle formation in mitosing cells and causes arrest at metaphase. Vinca alkaloids also inhibit other cellular activities that involve microtubules, such as leukocyte phagocytosis and chemotaxis as well as axonal transport in neurons. Side effects of the vinca alkaloids such as their neurotoxicity may be due to disruption of these functions.

- [Antineoplastic Agents](#)

VIP

- [Vasoactive Intestinal Peptide](#)

VIPomas

VIPoma stands for vasoactive intestinal polypeptide-secreting tumour. VIPomas are rare neuroendocrine tumours located in the pancreas.

► Somatostatin

Viral Proteases

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Synonyms

Viral proteinases

Definition

Viral proteases are enzymes (endopeptidases EC 3.4.2) encoded by the genetic material (DNA or RNA) of viral pathogens. The role of these enzymes is to catalyze the cleavage of specific peptide bonds in viral polypeptide precursors or in cellular proteins. In most cases these proteolytic events are essential for the completion of the viral infectious cycle. Viral proteases may use different catalytic mechanisms involving either serine, cysteine or aspartic acid residues to attack the scissile peptide bond. This bond is often located within conserved sequence motifs extending for up to ten residues. Selective recognition of these sequence patterns by a complementary substrate binding site of the enzyme ensures a high degree of specific recognition and cleavage (Fig. 1).

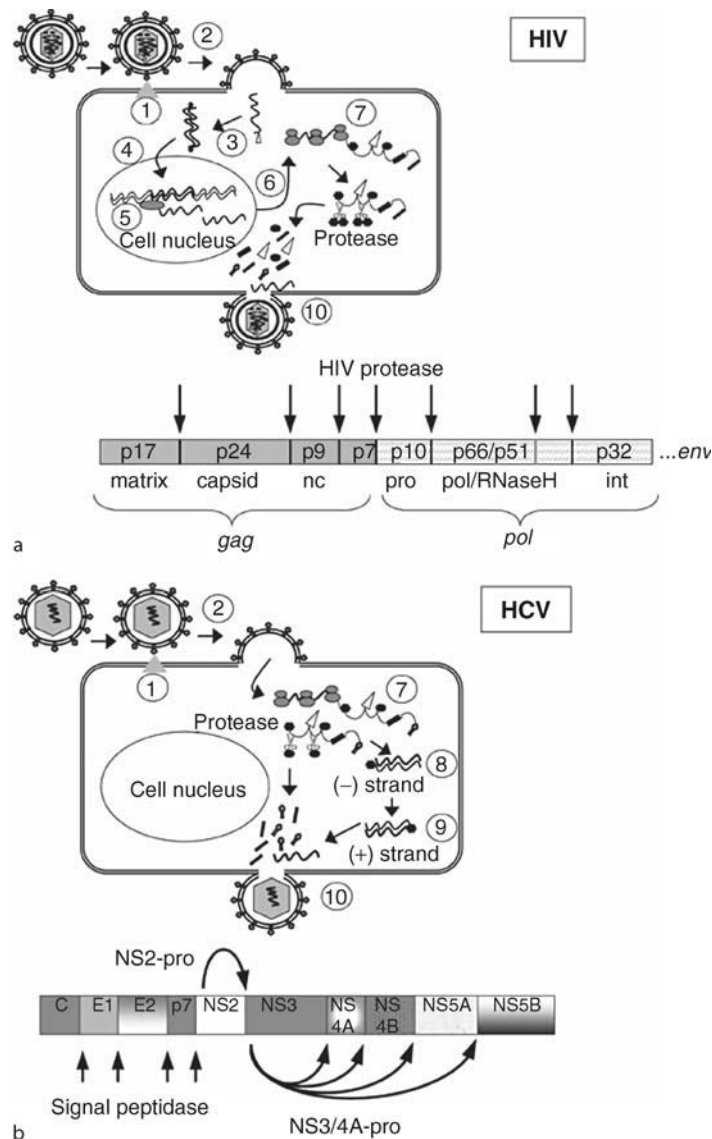
Basic Characteristics

The majority of approved antiviral pharmaceuticals belong to the category of nucleoside analogs that target viral polymerases. Adverse side effects, moderate clinical efficacy and especially the rapid emergence of drug-resistant mutant strains limits the efficacy of these inhibitors in monotherapy of chronic viral infections. Proteolytic maturation of large precursor proteins, catalyzed by virally encoded proteases, is a widespread strategy especially in viruses using RNA as genetic material. Since 1995, protease inhibitors have been successfully added to the therapeutic regimens of ►HIV infected individuals, and protease inhibitors are presently being developed to control other chronic viral infections such as ►hepatitis C (HCV). The infectious cycles of a retrovirus (such as the human immunodeficiency virus HIV), and of a (+)-strand-RNA virus (such as the

hepatitis C virus, HCV) are schematically shown below. Both viruses use RNA as genetic material but in contrast to (+)-strand RNA viruses, retroviral genomes are back-transcribed into a double stranded DNA molecule by a reverse transcriptase contained inside the viral particle and delivered to the cell during infection (►reverse transcription).

The HIV genome is organized into three major coding elements called *gag*, *pol* and *env* [1]. Upon integration of the provirus into the host cell genome, multiple copies of viral RNA are produced by hijacking the cellular transcription machinery. This RNA may have one of three different fates: A fraction of viral RNAs is reserved as genomes for new viral particles. Another fraction is spliced to yield subgenomic mRNA species that will be translated on membrane-bound polysomes to give rise to the *env* protein gp160, which is subsequently cleaved by cellular proteases into the envelope glycoproteins gp120 and gp41. Finally, a third fraction is used as mRNA for *gag* and *pol*. These gene products are produced as a nested set of precursor polypeptides such that the relative amount of *gag-pol* peptides (p160) is only 5% as abundant as *gag* peptides (p55). This relationship is maintained by partial bypassing of a translational stop signal at the end of *gag*. The *pol* gene encodes the viral enzymes protease, reverse transcriptase and ►integrase. Following a not well-understood autocatalytic event, the protease is released and performs nine different cleavages in the *gag* and *gag-pol* precursor proteins. Mutations in the protease lead to the generation of defective, noninfectious particles, demonstrating the essential role of this enzyme in the viral infectious cycle. The HIV protease belongs to the family of aspartic acid proteases (E.C.3.4.23) and is closely related to cellular enzymes such as renin, cathepsin D or pepsin. The structure of the HIV protease has been extensively studied by X-ray crystallography both, in its free form and complexed to inhibitors. It is a homodimer consisting of two identical polypeptide chains of 99 residues each. Each monomer contributes one catalytic aspartic acid residue in the active site, which is located at the dimer interface. Well-defined subsites and two flexible flap regions protruding over the active site contribute to specific recognition of substrates and inhibitors.

Reverse transcriptase and protease inhibitors interfere at different stages within the viral replication cycle. The former class of compounds (nucleoside analogs or nonnucleoside inhibitors) blocks infection at an early step prior to integration of the retro-transcribed viral genome into the genome of the host cell. Consistent with this mode of action, reverse transcriptase inhibitors are ineffective in arresting the spread of viruses from cells that are already infected. This is in contrast to protease inhibitors that act at a late stage of the infectious cycle. Many present therapeutic regimens simultaneously target both HIV enzymes by combining protease inhibitors with



Viral Proteases. Figure 1 Role of virally encoded proteases in the replication cycle of a retrovirus (HIV, part a) and of a (+)-strand RNA virus (HCV, part b). The numbers correspond to the following steps in the infectious cycle: (1) binding to cell surface receptor; (2) fusion; (3) reverse transcription; (4) integration; (5) transcription by host cell RNA polymerase; (6) RNA export and splicing; (7) translation; (8) (-)-strand synthesis; (9) (+)-strand synthesis; (10) assembly and budding of new viral particle. The proteolytic cleavage sites in the HIV gag-pol precursor and in the HCV polyprotein are schematically shown.

nucleoside analogs such as AZT, ddC, ddI, d4T or 3TC or nonnucleoside reverse-transcriptase inhibitors (nevirapine, efavirenz, delavirdine).

The infectious cycle of a (+)-strand RNA virus such as the hepatitis C virus differs by the fate of the viral RNA genome in the infected cell. Upon entry into the cell, the HCV genome is used as a messenger RNA to drive the synthesis of a large polyprotein precursor of about 3,000 residues [2]. The structural proteins are excised from the precursor by host cell signal peptidase.

The nonstructural region of the precursor, harboring the viral replication machinery, is cut into its mature components in a maturation reaction in which two viral proteases (NS2-pro and NS3/4A-pro) cooperate. Site-directed mutagenesis of an otherwise infectious cDNA has shown that both HCV-encoded proteases are necessary for viral infectivity, but most of the attention has so far been focused on one of them; a member of the serine protease family (EC 3.4.21) located in the N-terminal region of the viral NS3 protein.

The proteolytic activity of NS3 is turned on only upon binding to the cofactor NS4A, generating the active NS3/4A heterodimeric protease, and is used to perform a total of four different cleavages. The mature viral RNA-dependent RNA polymerase, the protein NS5B, also arises from one of these processing events. This enzyme subsequently replicates the viral genome in a two-step process involving a (–)-strand intermediate.

In contrast to retroviruses, proteolysis is an early event in the replication cycle of (+)-strand RNA viruses and both protease and polymerase inhibitors can be expected to halt the propagation of infectious viral particles from already infected cells.

The three-dimensional structure of the NS3 protease, either alone or in complex with its NS4A cofactor, was solved by both X-ray crystallography and by NMR spectroscopy. The enzyme adopts a chymotrypsin-like fold with two β -barrel domains contributing the residues that make up the catalytic triad; a histidine an aspartic acid and a serine residue. Comparison of structures obtained in the presence or in the absence of the cofactor lead to the conclusion that NS4A serves to stabilize the fold of the N-terminal beta-barrel of the protease thereby promoting the correct positioning of the catalytic machinery. In striking contrast to the HIV protease, there are no pronounced pockets or cavities in the proximity of the active site of NS3 that could serve to anchor inhibitors or substrates. Nevertheless, this protease is highly specific in binding to and processing of peptides with a consensus sequence spanning over ten residues. Specificity is conferred to this molecular recognition event by a series of weak interactions which are dispersed along a very extended interaction surface that involve hydrogen bonds, hydrophobic interactions and a crucial electrostatic complementarity between enzyme and active site ligand.

Drugs

HIV Protease Inhibitors

So far, five different protease inhibitors have been approved by the FDA for the treatment of HIV infection [3, 4]. Clinical trials in which protease inhibitors were evaluated in monotherapy demonstrated the potency of this class of inhibitors (decrease in HIV RNA levels, increase in CD4 cell counts). Treatment regimens were subsequently broadened to include reverse transcriptase inhibitors in combination with protease inhibitors. The result of these clinical trials has led to a list of guidelines with recommendations for the optimal treatment options. Prolonged control of the infection with combination therapy (highly active antiretroviral therapy, “HAART”) could be shown.

Saquinavir (SQV, Invirase)

Saquinavir was the first HIV protease inhibitor to obtain FDA approval in 1995 for the treatment of HIV infection

in combination with nucleoside analogs. Phase I clinical trials revealed low oral bioavailability due to limited gastro-intestinal absorption and extensive first-pass metabolism by human intestinal cytochrome P₄₅₀ 3A4 (CYP3A4) that may be limited by concomitant administration of CYP3A4 inhibitors (indinavir, ritonavir, ketoconazole, troleanomycin). Pharmacokinetics could also be improved by administration with a high-fat diet or in a novel soft gelatin capsule formulation (Fortovase). Saquinavir is generally well tolerated with mainly gastrointestinal side effects, including diarrhea, nausea and abdominal discomfort.

Ritonavir (RTV, Norvir)

Clinical evaluation of Ritonavir demonstrated good absorption from the gastrointestinal tract and high plasma drug levels. When given in combination with reverse transcriptase inhibitors, Ritonavir showed significant reduction of disease progression in clinical efficacy trials enrolling patients with advanced HIV disease, as compared to therapy with reverse transcriptase inhibitors alone. Ritonavir has a high affinity for several cytochrome P₄₅₀ isoenzymes and is a potent inhibitor of CYP3A4. This limits the concurrent use of ritonavir with agents that are metabolized by CYP3A such as various analgetics, antiarrhythmic agents, antibiotics, anticoagulants, anticonvulsants, antiemetics and antifungal agents. While such interactions may be clinically detrimental, simultaneous administration of low doses of Ritonavir was shown to boost plasma levels of other HIV protease inhibitors allowing for lower dosages or increasing dosing intervals. The most common adverse effects of Ritonavir are nausea, diarrhea, vomiting, muscular weakness, taste disturbance, anorexia, abnormal functioning of tissue and abdominal pain.

Indinavir (IDV, Crixivan)

Indinavir is a modified hydroxyethylamine peptidomimetic developed by rational drug design. In clinical trials, Indinavir was shown to significantly lower HIV RNA levels in plasma and to increase CD4 cell counts in both monotherapy and in combination drug regimens. Indinavir is generally well tolerated and in phase II clinical studies less than 6% of subjects taking Crixivan alone discontinued therapy due to drug-related adverse experiences. Nephrolithiasis was reported in 12.4% of adults and 29% of children, during clinical trials involving patients on Crixivan. As with other protease inhibitors, changes in body fat, increased bleeding in some patients with hemophilia, and increased blood sugar levels or diabetes have been reported. Additionally, severe muscle pain and weakness have occurred in patients also taking cholesterol-lowering medicines called “statins.”

Nelfinavir (NFV, Viracept)

Nelfinavir is a nonpeptidic, rationally designed HIV protease inhibitor. Nelfinavir has demonstrated efficacy in both monotherapy and in combination with the reverse transcriptase inhibitors stavudine (d4T) or zidovudine + 3TC. The major side effect is mild to moderate diarrhea.

Amprenavir (APV, Agenerase)

Amprenavir (APV, Agenerase) is the most recently approved HIV protease inhibitor. It is smaller and stereochemically less complex than the other drugs in this class. Adsorption of this compound was found to be impaired by high fat meals. Common side effects of Amprenavir are nausea, vomiting, diarrhea, rash and a tingling sensation around the mouth.

As with reverse transcriptase inhibitors, resistance to protease inhibitors may also occur. Mutations in the HIV protease gene were shown to confer resistance to each of the aforementioned molecules. In addition, passaging of virus in the presence of HIV protease inhibitors also gave rise to strains less susceptible to the original inhibitor or cross-reactive to other compounds in the same class.

New Drug Targets

Hepatitis C Virus Serine Protease [5]

Hepatitis C is a predominantly chronic infection affecting 1–3% of the world population. The infectious cycle of the hepatitis C virus has been outlined above. Inhibitors against the key viral protease, the NS3/4A serine protease are currently in clinical trials. This enzyme proved to be a particularly difficult target due to the peculiar architecture of its substrate recognition site (see above). An unusual feature of the NS3 protease is the property to undergo potent inhibition by its cleavage products. This property was exploited to generate a family of highly active peptide inhibitors that may serve as a basis for the development of peptidomimetics with improved pharmacokinetic properties. Also, mechanism-based inhibitors are being exploited.

Rhinovirus Protease [3]

Rhinoviruses, which represent the single major cause of common cold, belong to the family of picornaviruses that harbors many medically relevant pathogens. Inhibitors of the 3C protease, a cysteine protease, have shown good antiviral potential. Several classes of compounds were designed based on the known substrate specificity of the enzyme. Mechanism-based, irreversible Michael-acceptors were shown to be both potent inhibitors of the purified enzyme and to have antiviral activity in infected cells.

► Antiviral Drugs

► Non-viral Proteases

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Viral Vectors

► Gene-Therapy Vectors

Virostatics

► Antiviral Drugs

Virus-like Particle

A particle that spontaneously assembles from viral coat proteins in the absence of other viral components. Virus-like particles (VLPs) generated from recombinant coat proteins are used in vaccines against hepatitis B virus and papillomavirus.

► Biologicals

Vitamin A

Vitamin A describes a group of substances (retinol, retinyl esters, and retinal) with defined biological

activities. Certain metabolites of vitamin A, such as all-trans and cis-isomeric retinoic acids, can perform most of the biological functions of vitamin A; they are incapable of being metabolically reversibly converted into retinol, retinal, etc. The unit of measurement is the Vitamin A International Unit (IU) which measures growth in rat pups (1 mg retinol = 3333 IU Vitamin A).

► Retinoids

Vitamin B1

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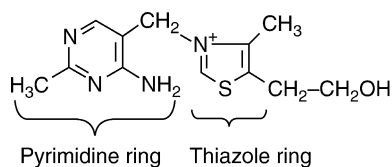
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Synonym

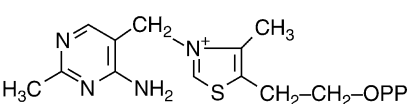
Thiamin (3-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium)

Definition

Free thiamin is a base that is stable under acidic conditions up to pH 7, moderately light-sensitive and susceptible to oxidative degradation as well as inactivation by irradiation. For use in pharmaceutical and other preparations the vitamin is handled in the form of solid water-soluble thiazolium salts (thiamin chloride hydrochloride, thiamin mononitrate). There are also synthetic lipophilic derivatives of the vitamin, termed allthiamins. They can pass biological membranes more easily and in a nearly dose-linear fashion. Due to their improved bioavailability they can be used to build up high thiamin stores in certain target organs (drug targeting). In the human body, thiamin concentration is highest in the heart, followed by kidney, liver, and brain. Yeast, pulses, wholemeal cereals, nuts, and pork are good dietary sources for thiamin [1].



Thiamin



Thiaminpyrophosphate

Some kinds of fish and crustacea contain thiaminases. These enzymes cleave thiamin and thus inactivate the vitamin. Some plant phenols, e.g., chlorogenic acid, may possess antithiaminic properties, too, though their mechanism of action is so far not well understood.

Figure 1 shows the chemical structure of vitamin B1 or thiamin (3-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium) and its coenzyme form thiaminpyrophosphate (TPP).

Mechanism of Action

Several enzymes of the intermediary metabolism require thiaminpyrophosphate (TPP, Fig. 1) as coenzyme, e.g., enzymes of the pyruvate dehydrogenase complex, α -ketoglutarate dehydrogenase complex, or pentose phosphate pathway.

TPP-dependent enzymes are involved in oxidative decarboxylation of α -keto acids, making them available for energy metabolism. Transketolase is involved in the formation of NADPH and pentose in the pentose phosphate pathway. This reaction is important for several other synthetic pathways. It is furthermore assumed that the above-mentioned enzymes are involved in the function of neurotransmitters and nerve conduction, though the exact mechanisms remain unclear.

Clinical Use (Including Side Effects)

Thiamin has a very low toxicity (oral LD₅₀ of thiaminchloride hydrochloride in mice: 3–15 g/kg body weight). The vitamin is used therapeutically to cure polyneuropathy, ►beriberi (clinically manifest thiamin deficiency), and Wernicke–Korsakoff Syndrome (►Wernicke encephalopathy and ►Korsakoff psychosis). In mild polyneuropathy, 10–20 mg/d water-soluble or 5–10 mg/d lipid-soluble thiamin are given orally. In more severe cases, 20–50 mg/d water-soluble or 10–20 mg/d lipid-soluble thiamin are administered orally. Patients suffering from ►beriberi or from early stages of Wernicke–Korsakoff Syndrome receive 50–100 mg of thiamin two times a day for several days subcutaneously or intravenously until symptoms are alleviated. Afterwards, the vitamin is administered orally for several weeks.

Vitamin B1. Figure 1 Structure of thiamin and its coenzyme form thiaminpyrophosphate (TPP).

- Vitamin E
- Vitamin K
- Folic Acid
- Niacin
- Pantothenic Acid
- Biotin
- Vitamin B2
- Vitamin B6
- Vitamin B12
- Vitamin C

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Vitamin B2

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Synonym

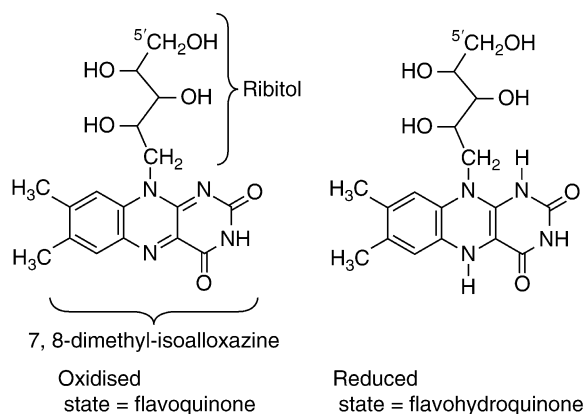
Riboflavin

Definition

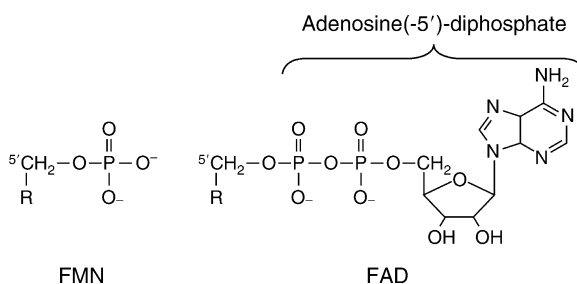
Vitamin B2 or riboflavin is chemically defined as 7,8-dimethyl-10-(1Y-D-ribityl)isoalloxazine. **Figure 1** shows the oxidized and reduced form of the vitamin. The ending “flavin” (from the latin word flavus = yellow) refers to its yellowish color.

Riboflavin is heat-stable in the absence of light, but extremely photosensitive. It has a high degree of natural fluorescence when excited by UV light. This property can be used for detection and determination. Two coenzymes (**Fig. 2**), flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), are derived from riboflavin.

Milk, milk products, and foods of animal origin contain high amounts of (free) riboflavin with good bioavailability. In foods of plant origin, the majority of riboflavin is protein-bound and therefore less bioavailable. Cereal germs and bran are plant sources rich in riboflavin [1].



Vitamin B2. Figure 1 Structure of oxidized and reduced riboflavin.



Vitamin B2. Figure 2 Structure of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

Mechanism of Action

The formation of FMN and FAD is ATP-dependent and takes place predominantly in liver, kidney, and heart. It is controlled by thyroid hormones [2].

So far, about 60 flavoenzymes have been identified, the majority containing FAD. Examples for flavoenzymes are the mitochondrial enzymes of the respiratory chain, succinate dehydrogenase, L-gulonolactone oxidase, monoamino oxidase, xanthinoxidase, thioredoxin reductase, and glutathione reductase. Flavin coenzymes are involved in both one- and two-electron transfer reactions and catalyze hydroxylations, oxidative decarboxylations, dioxygenations as well as reductions of oxygen to hydrogen peroxide. Through their functions as coenzymes, FMN and FAD are involved in the metabolism of glucose, fatty acids, amino acids, purines, drugs and steroids, folic acid, pyridoxin, vitamin K, niacin, and vitamin D.

The FAD-dependent enzyme glutathione reductase plays a role in the antioxidant system. Glutathione reductase restores reduced glutathione (GSH), the most important antioxidant in erythrocytes, from oxidized glutathione (GSSG) [1, 2].

Clinical Use (Including Side Effects)

Up to date, no case of riboflavin intoxication has been described in the literature and riboflavin intake is considered safe even at higher doses.

The vitamin is administered therapeutically to reverse riboflavin deficiency symptoms or to prevent deficiency in high risk groups. Among the high risk groups to develop riboflavin deficiency count persons who regularly take certain drugs (e.g., antidepressants, oral ►[contraceptives](#)), malnourished patients, patients after trauma, patients suffering from malabsorption, and chronic alcoholics. The doses given are >10 mg/d. Two other groups who benefit from riboflavin supplementation are newborns with hyperbilirubinemia who are treated with phototherapy. The supply of 0.5 mg riboflavin/kg body weight and day can here accelerate the photodegradation of bilirubin. Furthermore, persons with congenital methemoglobinemia might profit from the intake of high doses of riboflavin (20–40 mg/d) [2].

- Folic Acid
- Niacin
- Pantothenic Acid
- Vitamin B1
- Biotin
- Vitamin B6
- Vitamin B12
- Vitamin C

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1. Biesalski HK, Grimm P (2005) Pocket Atlas of Nutrition. Thieme, New York
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Vitamin B6

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Definition

Vitamin B6 ([Fig. 1](#)) is the collective name for all 3-hydroxy-2-methylpyridine-derivatives with vitamin

B6 function, including pyridoxin (= PN, alcohol), pyridoxal (= PL, aldehyde), pyridoxamine (= PM, amine), and their 5'-phosphorylated forms.

In general, pyridoxamine and pyridoxin are more stable than pyridoxal. All vitamers are relatively heat-stable in acid media, but heat labile in alkaline media. All forms of vitamin B6 are destroyed by UV light in both neutral and alkaline solution. The majority of vitamin B6 in the human body is stored in the form of pyridoxal phosphate in the muscle, bound to glycogen phosphorylase.

Plants contain to some extent less bioavailable forms of vitamin B6, e.g., glycosylates, or biologically inactive metabolites, e.g., ε-pyridoxin-lysine-complexes. In addition, the release of vitamin B6 from foods rich in fiber is assumed to be delayed. The bioavailability of vitamin B6 from animal-derived foods is therefore overall higher than from plant-derived foods. Good dietary sources of vitamin B6 include chicken, fish, pork, beans, and pulses [1].

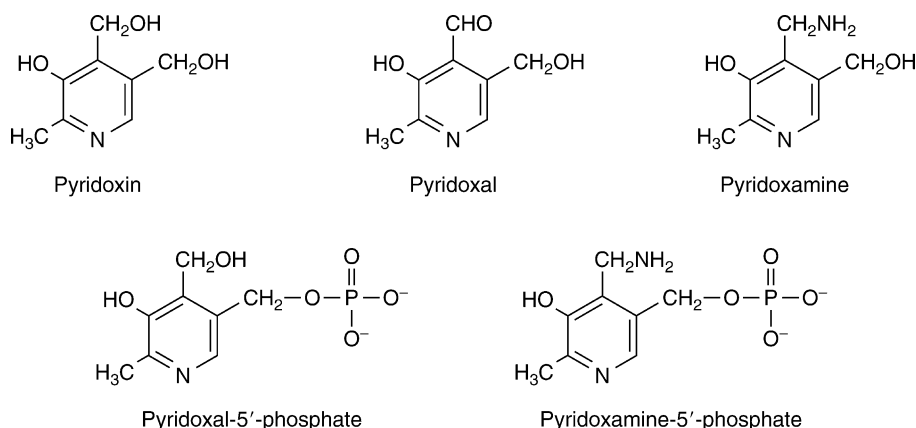
Mechanism of Action

Pyridoxal phosphate mainly serves as coenzyme in the amino acid metabolism and is covalently bound to its enzyme via a Schiff base. In the enzymatic reaction, the amino group of the substrate and the aldehyde group of PLP form a Schiff base, too. The subsequent reactions can take place at the α-, β-, or γ-carbon of the respective substrate. Common types of reactions are decarboxylations (formation of biogenic amines), transaminations (transfer of the amino nitrogen of one amino acid to the keto analog of another amino acid), and eliminations.

Pyridoxamine phosphate serves as a coenzyme of transaminases, e.g., lysyl oxidase (collagen biosynthesis), serine hydroxymethyl transferase (C1-metabolism), δ-aminolevulinic synthase (porphyrin biosynthesis), glycogen phosphorylase (mobilization of glycogen), aspartate aminotransferase (transamination), alanine aminotransferase (transamination), kynureninase (biosynthesis of niacin), glutamate decarboxylase (biosynthesis of GABA), tyrosine decarboxylase (biosynthesis of tyramine), serine dehydratase (β-elimination), cystathionine β-synthase (metabolism of methionine), and cystathionine γ-lyase (γ-elimination).

Vitamin B6-coenzyme is involved in a variety of reactions, e.g., in the immune system, gluconeogenesis, erythrocyte function, niacin formation, nervous system, lipid metabolism, and in hormone modulation/gene expression [1, 2].

- Folic Acid
- Niacin
- Pantothenic Acid
- Vitamin B1
- Vitamin B2



Vitamin B6. Figure 1 Structure of pyridoxin, pyridoxal, pyridoxamine, and the coenzymes pyridoxal-5'-phosphate and pyridoxamine-5'-phosphate.

- Biotin
- Vitamin B12
- Vitamin C

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Vitamin B12

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Definition

Vitamin B12 (Fig. 1) is defined as a group of cobalt-containing corroids known as cobalamins. The common features of the vitamers are a corrin ring (four reduced pyrrole rings) with cobalt as the central atom, a nucleotide-like compound and a variable ligand. Vitamin B12 is exceptional in as far as it is the only vitamin containing a metal-ion. The vitamers present in biological systems are hydroxo-, aquo-, methyl-, and 5'-deoxyadenosylcobalamin.

Only microorganisms are able to synthesize vitamin B12. Microbiological vitamin B12 synthesis in humans takes place, however, in the lower intestine where the

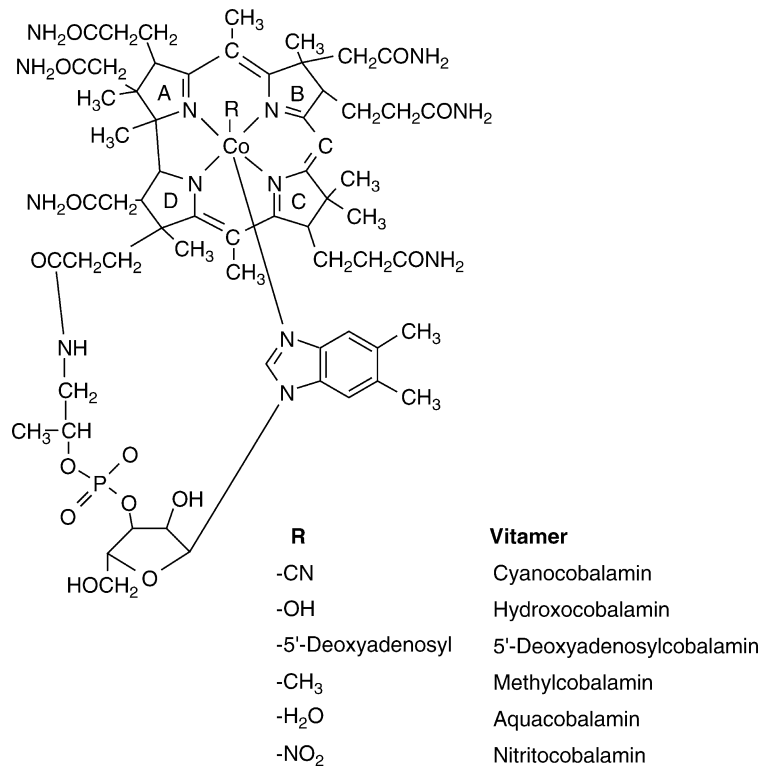
body cannot sufficiently absorb the vitamin. Therefore, humans have to supply the vitamin via the diet. The vitamers differ in their photosensitivity, but they are all sooner or later inactivated when exposed to visible light. Good dietary sources of vitamin B12 comprise mostly animal-derived products, e.g., liver, meat, egg (yolk), milk and milk products, whereas vegan (►vegetarian) diets are basically vitamin B12-free [1].

Vitamin B12 is special in as far as its absorption depends on the availability of several secretory proteins, the most important being the so-called intrinsic factor (IF). IF is produced by the parietal cells of the fundic mucosa in man and is secreted simultaneously with HCl. In the small intestine, vitamin B12 (extrinsic factor) binds to the alkali-stable gastric glycoprotein IF. The molecules form a complex that resists intestinal proteolysis. In the ileum, the IF-vitamin B12-complex attaches to specific mucosal receptors of the microvilli as soon as the chymus reaches a neutral pH. Then either cobalamin alone or the complex as a whole enters the mucosal cell.

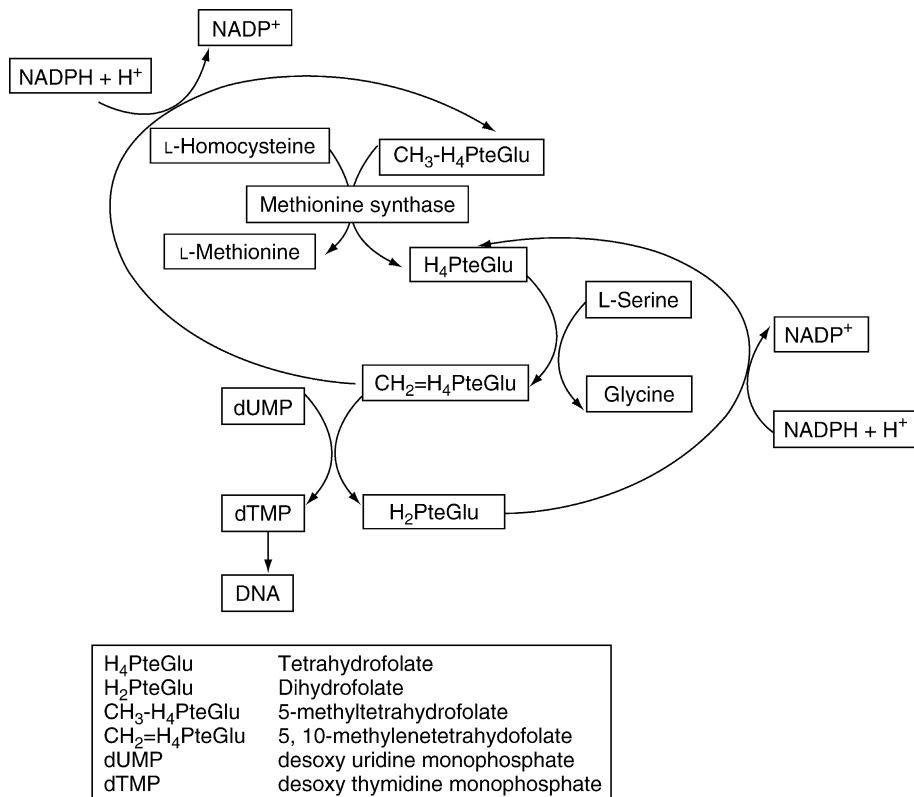
Mechanism of Action

Vitamin B12 appears in two coenzymatic forms, namely methylcobalamin (cytosol) and 5'-deoxyadenosylcobalamin (mitochondria). Vitamin B12-dependent enzymes are [1]

1. Methylmalonyl-CoA mutase: 5'-deoxyadenosylcobalamin is part of dimethylbenzimidazolecobamide coenzyme, a constituent of methylmalonyl-CoA mutase. This mutase catalyses the isomerization of methylmalonyl-CoA to succinyl-CoA (anaplerotic reaction of the citric acid cycle).
2. Leucine 2,3-amino-mutase.
3. N5-Methyltetrahydrofolate homocysteine methyltransferase (= methionine synthase). This reaction is essential to restore tetrahydrofolate from N5-methyltetrahydrofolate (Fig. 2).



Vitamin B12. Figure 1 Structure of the different vitamin B12 vitamers.



Vitamin B12. Figure 2 Selected reactions in which folic acid coenzymes are involved.

Clinical Use (Including Side Effects)

Vitamin B12 deficiency develops when the availability of intrinsic factor is reduced (atrophic gastritis, gastric mucosal defects, after gastrectomy), in persons with malabsorption syndromes, especially when the terminal ileum is involved, in breastfed infants whose mothers have lived on a vegan (refer to ►[vegetarian](#)) diet for years, in persons with congenital abnormalities in cobalamin metabolism, and in persons having intestinal parasites, especially fish tapeworms (*Diphyllobothrium latum*). The characteristic symptoms of vitamin B12 deficiency include macrocytic hyperchromic ►[anemia](#) (common symptom in both vitamin B12 and folic acid deficiency) and ►[funicular myelitis](#) (neurological disorder, characteristic of vitamin B12 deficiency) [1, 2].

Cyano- and hydroxocobalamin – both can be converted to the physiologically relevant coenzymes methyl- and 5'-deoxyadenosylcobalamin in the liver – are used for therapeutical applications. When pernicious anemia caused by chronic atrophic gastritis has been diagnosed, it is treated as follows: During the first 7 days of treatment, 1000 µg of hydroxocobalamin/d are administered parenterally, usually intramuscularly. Then, the same dose is given once weekly for 4–6 weeks. The aim is to alleviate the deficiency symptoms and at the same time to replenish the stores. Afterwards, 1000 µg hydroxocobalamin should be given parenterally every 2 months lifelong to avoid relapse [1, 2].

When ►[funicular myelitis](#) occurs in advanced stages of vitamin B12 deficiency, patients are given 250 µg vitamin B12/d during the first 2 weeks of treatment to alleviate the symptoms and to replenish the stores. If the deficiency has been caused by disturbed vitamin B12 absorption, lifelong monthly injections of 100 µg vitamin B12 are indicated [2].

Whether supplementation of vitamin B12 is useful in the therapy of a number of neurological disorders is still subject to discussion and further investigations.

- [Folic Acid](#)
- [Niacin](#)
- [Pantothenic Acid](#)
- [Vitamin B1](#)
- [Vitamin B2](#)
- [Vitamin B6](#)
- [Biotin](#)
- [Vitamin C](#)

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Vitamin C

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Synonym

L-ascorbic acid

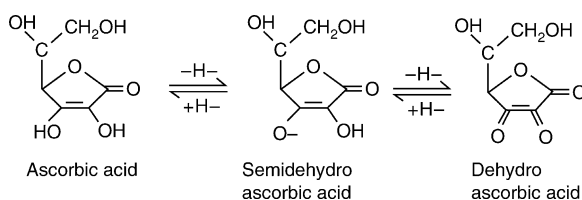
Definition

Vitamin C or L-ascorbic acid ([Fig. 1](#)) is chemically defined as 2-oxo-L-theo-hexono-4-lactone-2,3-enediol. Ascorbic acid can be reversibly be oxidized to semidehydro- L-ascorbic acid and further to dehydroascorbic acid.

Ascorbic acid is photosensitive and unstable in aqueous solution at room temperature. During storage of foods, vitamin C is inactivated by oxygen. This process is accelerated by heat and the presence of catalysts. Ascorbic acid concentration in human organs is highest in adrenal and pituitary glands, eye lens, liver, spleen, and brain. Potatoes, citrus fruits, black currants, sea buckthorns, acerola, rose hips, and red paprika peppers are among the most valuable vitamin C sources [1, 2].

Mechanism of Action

Ascorbic acid scavenges reactive oxygen and nitrogen species. The resulting ascorbate radical converts quickly to ascorbic acid and dehydroascorbic acid, but does usually react with other surrounding molecules. This is why ascorbic acid is such an important physiological antioxidant. Dehydroascorbic acid is enzymatically reduced to ascorbic acid in biological systems. This recycling helps to maintain the ascorbic acid stores in the tissue at a high level. In addition to its antioxidant function, ascorbic acid plays a role as a cofactor of mono- and dioxygenases in norepinephrine synthesis, hormone activation, collagen biosynthesis,



Vitamin C. Figure 1 Structure of ascorbic acid, semidehydroascorbic acid, and dehydroascorbic acid.

carnitine biosynthesis, and tyrosine metabolism (24). Furthermore, ascorbic acid improves intestinal absorption of inorganic iron by reducing Fe^{3+} to Fe^{2+} (only the latter has a high affinity to the mucosal iron receptor). In the stomach, ascorbic acid inhibits the formation of nitrosamines and thus might be important in protecting the stomach from ulcers and ►cancer. Finally, ascorbic acid competitively inhibits the glycosylation of proteins, a process that could be important for the long-term prognosis of diabetes (►diabetes mellitus) [2].

Vitamin C status is supposed to play a role in immune function and to influence the progression of some chronic degenerative diseases like ►atherosclerosis, ►cancer, cataracts, and osteoporosis. The role of vitamin C in immune function, especially during common cold and upper respiratory tract infection, is the subject of lively debate. The exact mechanisms of action have not yet been fully elucidated, but the results of several trials point to a reduced duration and intensity of infections in subjects consuming high amounts of vitamin C (200–1000 mg/d). However, the incidence of common cold was not influenced significantly (24).

Ascorbic acid is able to regenerate ►vitamin E from tocopheryl radicals. This reaction might be important in the context of ►atherosclerosis prevention. The role of vitamin C in cancer prevention is still unclear. Its importance in the etiology of various types of ►cancer has not yet been proven. The observed high vitamin C intake levels in groups with low cancer rates might simply have been a marker for high fruit and vegetable intake [2].

Clinical Use (Including Side Effects)

Presently, the full clinical picture of vitamin C-deficiency, called scurvy, is rarely seen. The typical symptoms include mucosal and subcutaneous bleedings, follicular hyperkeratosis, and gingivitis. Current issues regarding this vitamin include the question where the optimal dose lies to gain optimum health benefits. A daily intake of 75 mg for adults is considered sufficient to prevent vitamin C deficiency symptoms. The RDA for adults was recently increased by 25–50%, from 60 to 75 mg for females and 90 mg for males, acknowledging the fact that increased doses might help prevent chronic diseases. The results of numerous studies suggest that optimum health benefits are achieved (i.e., maintenance of maximum plasma and tissue stores) at even higher daily intakes of about 100–200 mg/d [2].

Vitamin C is not toxic and a hypervitaminosis has not yet been described in the literature. The statement that high vitamin C intake increases the risk of kidney stones could not be maintained.

Vitamin C requirements are increased during pregnancy and lactation, in patients undergoing hemodialysis and in smokers. Seniors often have suboptimal intakes.

On the basis of available results, persons suffering from common cold might benefit from taking 1–2 g/d of vitamin C. However, the usefulness of taking high amounts of vitamin C to prevent common cold has yet to be proven [2].

So far, it is not possible to give any recommendations concerning the vitamin C intake required for the prevention of osteoporosis, cataracts, ►cancer, or cardiovascular disease.

- Folic Acid
- Niacin
- Pantothenic Acid
- Vitamin B1
- Vitamin B2
- Vitamin B6
- Vitamin B12
- Biotin
- Cancer
- Atherosclerosis
- Diabetes Mellitus

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Vitamin D

Summary term for a number of steroid hormones and their precursors with differentiation-inducing activity in many tissues. As regards bone, three components are relevant: cholecalciferol (“vitamin D”); 25-hydroxyvitaminD3 (calcidiol) and 1,25-dihydroxyvitamin D3 (calcitriol). The latter is the biologically active form and increases both intestinal calcium absorption and bone resorption. Vitamin D preparations are widely used for the treatment of osteoporosis. Daily supplementation with vitamin D reduces bone loss in postmenopausal women and hip fractures in elderly subjects.

- Bone Metabolism
- Ca²⁺ sensing Receptor

Vitamin E

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Synonyms

The term vitamin E describes a family of eight antioxidants, four tocopherols, alpha (α), beta (β), gamma (γ) and delta (δ), and four tocotrienols (also α , β , γ , and δ). α -Tocopherol is present in nature in only one form, RRR α -tocopherol. The chemical synthesis of α -tocopherol results in eight different forms (SRR, SSR, SRS, SSS, RSR, RRS, RSS, RRR), only one of which is RRR α -tocopherol. These forms differ in that they can be “right” (R) or “left” (S) at three different places in the α -tocopherol molecule. RRR α -tocopherol is the only form of vitamin E that is actively maintained in the human body and is therefore the form of vitamin E found in the largest quantities in the blood and tissue. A protein synthesized in the liver (α -TTP: alpha-tocopherol transfer protein) preferentially selects the natural form of vitamin E (RRR α -tocopherol) for distribution to the tissues. However, the mechanisms for the regulation of vitamin E in tissues are not known

Definition

In 1922, Evans and Bishop named the animal nutritional factor essential of reproduction “Vitamin E”. In the 1960s, vitamin E was associated with antioxidant function. Twenty-five years later, vitamin E has been found to possess functions that are independent of its antioxidant and free radical scavenging ability. α -Tocopherol specific molecular mechanisms were discovered which are still under investigation.

Mechanism of Action

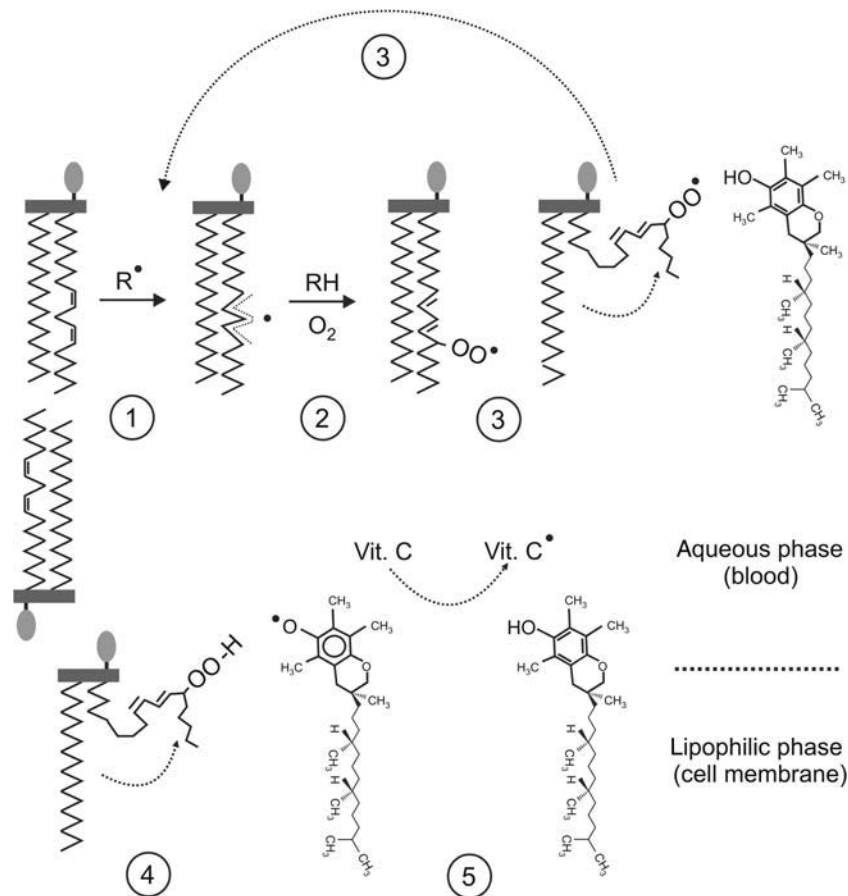
The main function of α -tocopherol in humans appears to be that of a non-specific chain-breaking antioxidant that prevents the propagation of lipid peroxidation (Fig. 1). ►Reactive oxygen species (ROS) are formed primarily in the body during normal metabolism and also upon exposure to environmental factors such as pollutants or cigarette smoke. Fats (first of all several fold polyunsaturated fatty acids; PUFAS), which are an integral part of all cell membranes, are vulnerable to destruction through oxidation by ROS. The fat-soluble vitamin, α -tocopherol, is uniquely suited to intercepting free radicals and preventing a chain reaction of

lipid destruction (Fig. 1). Aside from maintaining the integrity of cell membranes throughout the body, α -tocopherol also protects the fats in low density lipoproteins (LDL) from oxidation. Oxidized LDL have been implicated in the development of cardiovascular diseases. When a molecule of α -tocopherol neutralizes a free radical, it is altered in such a way that its antioxidant capacity is lost. However, other antioxidants, such as vitamin C, are capable of regenerating the antioxidant capacity of α -tocopherol. If the rate of oxidation is greater than the rate of regeneration, α -tocopherol concentrations in the body will decrease. Low levels of α -tocopherol have been associated with increased incidence of cardiovascular diseases (heart disease and stroke), cancer, cataracts, and immune function. Several other functions of α -tocopherol have been identified which likely are not related to its antioxidant capacity.

Potential, Non-Antioxidant, α -Tocopherol Specific Effects [1]

A specific role for vitamin E in a required metabolic function has not been found. In addition to its direct antioxidant effects, α -tocopherol has been reported to have specific molecular functions.

The proposed molecular mechanisms of α -tocopherol are associated with transcriptional and post-transcriptional events. Activation of diacylglycerol kinase and protein phosphatase 2A (PP₂A) and the inhibition of protein kinase C (PKC), cyclooxygenase-1, 5-lipoxygenase, and cytokine release by α -tocopherol are all examples of post-transcriptional regulation. PKC inhibition by α -tocopherol was reported to be involved in cell proliferation and differentiation in smooth muscle cells, human platelets, and monocytes. The inhibitory effect of α -tocopherol on PKC can be correlated to a dephosphorylation of PKC α . Dephosphorylation of PKC occurs via the protein PP₂A, which has been found to be activated in vitro by treatment of α -tocopherol. Genes affected by tocopherols were divided in five groups. Group 1: genes that are involved in the uptake and degradation of tocopherols: α -TTP, cytochrome P450 (CYP3A), gamma-glutamyl-cysteine synthetase heavy subunit, and glutathione-S-transferase. Group 2: genes that are implicated with lipid uptake and atherosclerosis: CD36, SR-BI, and SR-AI/II. Group 3: genes that are involved in the modulation of extracellular proteins: tropomyosin, collagen-alpha-1, MMP-1, MMP-19, and connective tissue growth factor. Group 4: genes that are connected to adhesion and inflammation: E-selectin, ICAM-1 integrins, glycoprotein IIb, IL-2, IL-4, IL-1b, and transforming growth factor-beta (TGF-beta). Group 5: genes implicated in cell signaling and cell cycle regulation: PPAR-gamma, cyclin D1, cyclin E, Bcl2-L1, p27, CD95 (APO-1/Fas ligand), and 5 α -steroid reductase



Vitamin E. Figure 1 The role of vitamin E in the mechanism of lipid peroxidation: (1) *Initiation*: a radical ($R^\bullet$) must be present; (2) *Hydrogen abstraction* from a weakened C - H bond on carbon atoms adjacent to double bonds in a PUFA, forming a carbon centred radical; (3) *Propagation*: rearrangement to a conjugated diene followed by reaction with molecular oxygen (O_2) produces a highly reactive peroxy radical which simultaneously propagates the reaction by hydrogen abstraction from another PUFA ($\rightarrow$ chain reaction); (4) *Termination*: hydrogen abstraction from α -tocopherol form a lipid hydroperoxide and a tocopheroxyl radical. The chain reaction is broken since α -tocopheroxyl radical will not react with an adjacent PUFA; (5) The α -tocopheroxyl radical can be recycled by vitamin C.

type 1. The transcription of p27, Bcl2, α -TTP, cytochrome P450 (CYP3A), gamma-glutamyl-cysteine synthetase heavy subunit, tropomyosin, IL-2, and CTGF appears to be upregulated by one or more tocopherols. All the other listed genes are downregulated

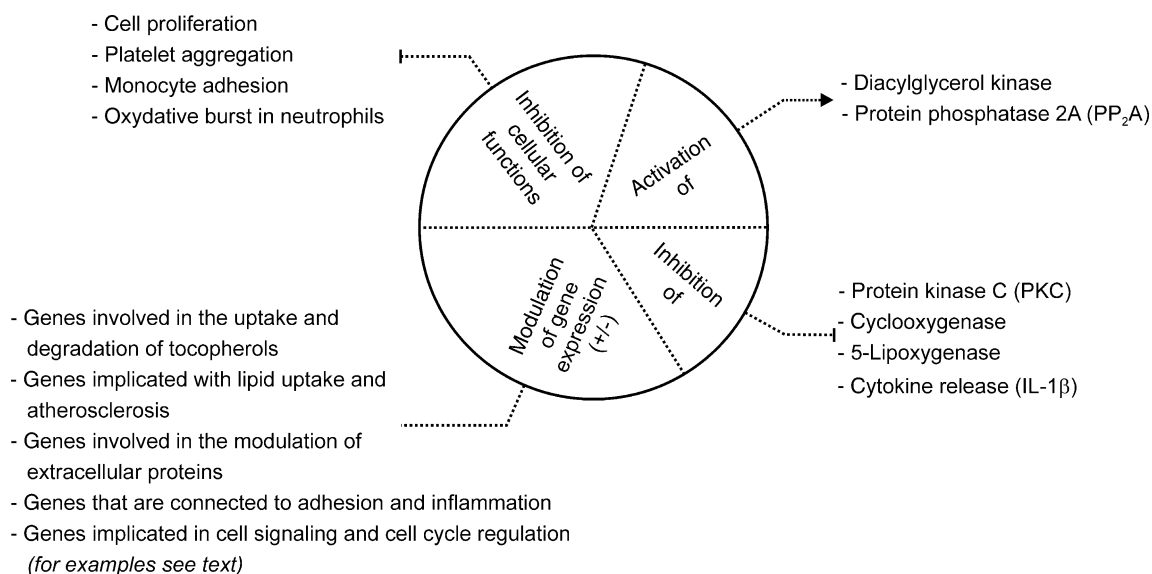
Gene regulation by tocopherols has mainly been associated with PKC because of its deactivation by α -tocopherol and its contribution in the regulation of a number of transcription factors (NF-kappaB, AP1). A direct participation of the pregnane X receptor (PXR)/retinoid X receptor (RXR) has been also shown. The antioxidant-responsive element (ARE) and the TGF-beta-responsive element appear in some cases to be implicated as well. The observed immunomodulatory function of α -tocopherol may also be attributed to the fact that the release of the proinflammatory cytokine interleukin-1 β can be inhibited by α -tocopherol via

inhibition of the 5-lipoxygenase pathway. In addition, the inhibition of PKC activity from monocytes by α -tocopherol is followed by inhibition of phosphorylation and translocation of the cytosolic factor p47 (phox) and impaired assembly of the NADPH-oxidize and of superoxide production. An overview of the non-antioxidant effects of α -tocopherol is summarized in Fig. 2.

Clinical Use (Disease Treatment)

Role in Cardiovascular Diseases

Low levels of vitamin E have been associated with increased incidence of coronary artery disease. Observational studies have therefore suggested that supplemental α -tocopherol might have value in the treatment of cardiovascular disease. Clinical studies demonstrated contradicting results regarding the benefits of vitamin E in the prevention of cardiovascular disease. Four



Vitamin E. Figure 2 Non-antioxidant properties of α -tocopherol.

large-scale, randomized, double-blind clinical intervention studies have tested the ability of vitamin E to prevent myocardial infarction. One was strongly positive, the other three were neutral. Therefore, the role of α -tocopherol supplementation in the treatment of cardiovascular disease is unclear and the results of further large intervention trials must be bided to clarify the role of vitamin E in the pathogenesis of cardiovascular diseases. In a recently published meta-analysis, the authors concluded that available data (peer-reviewed articles in MEDLINE from 1966 up to July 2005) do not support the supplementation of vitamin E in cardiovascular disease [2]. The authors of the extended HOPE (Heart Outcomes Prevention Evaluation) study even came to the conclusion that long-term vitamin E supplementation does not prevent major cardiovascular events and may increase the risk for heart failure.

Role in Diabetes Mellitus

It has been proposed that the development of the complications of **diabetes mellitus** may be linked to oxidative stress and therefore might be attenuated by antioxidants such as vitamin E. Furthermore, it is discussed that glucose-induced vascular dysfunction in diabetes can be reduced by vitamin E treatment due to the inactivation of PKC. Cardiovascular complications are among the leading causes of death in diabetics. In addition, a postulated protective effect of vitamin E (antioxidants) on fasting plasma glucose in type 2 diabetic patients is also mentioned but could not be confirmed in a recently published triple-blind, placebo-controlled clinical trial [3]. To our knowledge, up to now no clinical intervention trials have tested directly whether vitamin E can ameliorate the complication of diabetes.

Role in Alzheimer's Disease

Alzheimer's disease is a degenerative disease of the brain, in which oxidative stress appears to play a role. Vitamin E is thought to prevent brain cell damage by destroying toxic free radicals. In a 2-year, double-blind, placebo-controlled, randomized, multicentre trial, supplementation of 341 patients who had moderate neurological impairment with 2,000 IU synthetic α -tocopherol daily resulted in a significant slowing of the progression of Alzheimer's disease [4]. Although these results are promising, it is too early to draw any conclusions about the usefulness of vitamin E in Alzheimer's disease or other neurological diseases. In general, clinical studies demonstrated contradicting results regarding the benefits of vitamin E in these diseases with the exception of Alzheimer's disease, which seemed to show benefit when vitamin E was used. Therefore, further studies are required to determine the role of vitamin E supplementation in central nervous system disorders.

A recent review [5] concluded that there is little convincing evidence that vitamin E increases antioxidant defences in the body. This would also question a benefit of vitamin E in the prevention and management of the diseases mentioned above.

Friedreich Ataxia

Friedreich's ataxia (FRDA) is the most common inherited ataxia. FRDA is an autosomal recessive degenerative disorder caused by a GAA triplet expansion or point mutations in the *FRDA* gene on chromosome 9q13. Decreased mitochondrial respiratory chain function and increased oxidative stress have been implicated in the pathogenesis of FRDA, raising the possibility that energy enhancement and antioxidant therapies may be an

effective treatment. Pilot studies have shown the potential effect of antioxidant therapy in this condition and provide a strong rationale for designing larger clinical randomized trials. In the cases of Ataxia with vitamin E deficiency, high levels of vitamin E (800 IU) increased the blood concentration and stabilized the neurological signs especially in early stages of the disease. These patients lack a key protein in the liver (α -TTP) that is responsible for putting the vitamin E in the blood lipoproteins for transport to the tissues. For this reason, these patients develop serious deficiency which cause major neurological and muscle damage.

Side Effects

Due to bleeding risk, individuals on anticoagulant therapy or individuals who are vitamin K-deficient should not take vitamin E supplementation without close medical supervision. Absent of that, vitamin E is a well-tolerated relatively non-toxic nutrient. A tolerable upper intake level of 1,000 mg daily of α -tocopherol of any form (equivalent to 1,500 IU of RRR α -tocopherol or 1,100 IU of all-rac- α -tocopherol) would be, according to the Food and Nutrition Board of the Institute of Medicine, the highest dose unlikely to result in haemorrhage in almost all adults.

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Vitamin K

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Synonyms

Phylloquinones (vitamin K1, produced by plants) and menaquinones-n (MK-n, vitamin K2)

Definition

Vitamin K represents two groups of substances named “phyloquinones” (vitamin K1; produced by plants) and menaquinones-n (MK-n; vitamin K2). Menaquinones are synthesized by bacteria, using repeated 5-carbon units in the molecules side chain. n stands for the number of 5-carbon units. Interestingly, MK-4 is synthesized only in small amounts by bacteria but can be produced by animals (including humans) from phyloquinones and is found in a number of organs. For an overview see [1, 2].

Vitamin K belongs to the group of fat-soluble vitamins. The term “K” refers to its main actions in blood-clotting, coagulation, in german named “Koagulation”. In the coagulation cascade seven coagulation factors depend on Vitamin K which acts as coenzyme for a vitamin K-dependent carboxylase that catalyzes the carboxylation of glutamic acid. This specific carboxylation occurs only in a small number of proteins but is essential for their ability to bind calcium [5]. Vitamin K is light-sensitive but almost resistant to heat.

Vitamin K carboxylase is a transmembraneous protein in the lipid bilayer of the endoplasmatic reticulum (ER). It is highly glycosylated and its C-terminal is on the luminal side of the membrane. Besides its function as carboxylase it takes part as an epoxidase in the vitamin K cycle (Fig. 1). For the binding of the γ -carboxylase the vitamin K-dependent proteins have highly conserved special recognition sites. Most vitamin K-dependent proteins are carboxylated in the liver and in osteoblasts, but also other tissues might be involved, e.g., muscles.

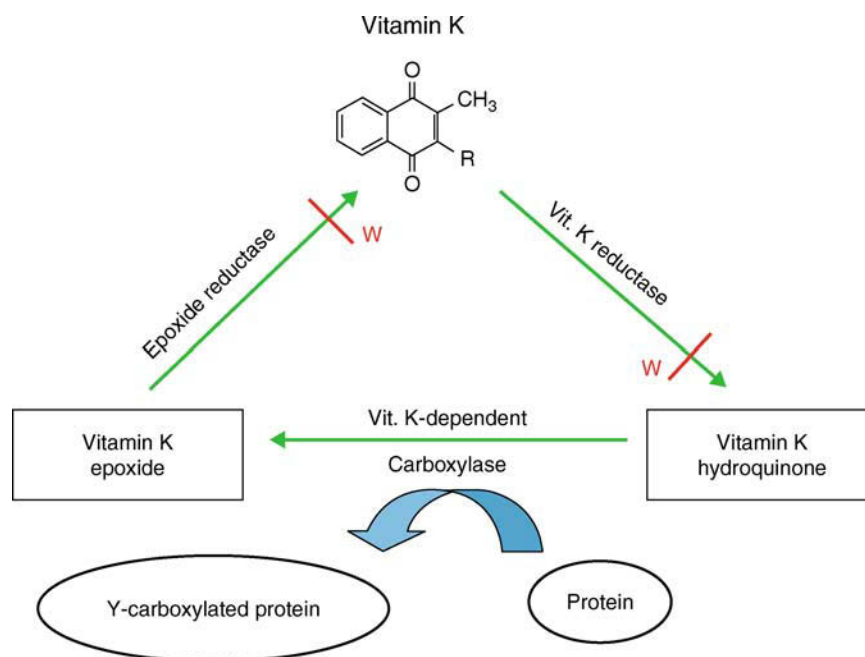
Mechanism of Action

General Aspects

Although vitamin K is a fat soluble vitamin, only little stores are found in the body which have to be refilled permanently via dietary input. The role of vitamin K derived from bacteria in the colon is controversially discussed, as the concentration of biliary acids for the resorption the fatsoluble vitamin K is very low in the colon. In addition, only diseases of the small intestine lead to a deficit in vitamin K concentration which cannot be restored by K2 production of colonic bacteria. However, watersoluble vitamin Ks can be resorbed by the colonic mucosa. Maybe because of the little stores for vitamin K, the process of vitamin K-dependent carboxylation of proteins is part of a cycle with several steps during which vitamin K normally is regenerated (see Fig. 1) and thus can be used several times.

Coagulation

Binding calcium ions (Ca^{2+}) is a prerequisite for the activation of seven clotting factors in the coagulation cascade that are dependent on vitamin K. The term cascade indicates, that the factors involved depend from



Vitamin K. Figure 1 The vitamin K cycle. w indicates where warfarin or other vitamin K antagonist inhibit the cycle.

each other to become activated and to fulfil their special part to stop bleeding. Prothrombin (factor II), factors VII, IX, and X play central roles in the coagulation cascade, protein Z seems to enhance the thrombin action via promoting its association with phospholipids in cell membranes. The proteins C and S (as a cofactor for C) act as anticoagulant proteins (they inhibit clotting factors Va and VIIIa) such providing an also vitamin K-dependent control and balance of the whole cascade. With both, coagulant and anticoagulant factors present in the cascade in the liver, uncontrolled bleeding and/or clotting can be well prevented. On the other hand, liver damage or diseases can disturb this well controlled cascade, e.g., via reduced clotting factors in the circulating blood resulting in uncontrolled bleeding (hemorrhage) [4] which in turn seem to be treatable by the application of recombinant factor seven (rFVIIa) [3].

Bone Mineralization

In bone, three proteins have been described which are vitamin K-dependent, osteocalcin (bone Gla protein), matrix Gla protein (MGP), and protein S. Osteocalcin is synthesized by osteoclasts, regulated by the active form of vitamin D, calcitriol. Its capacity to bind calcium needs a vitamin K-dependent γ -carboxylation of three glutamic acid residues. The calcium binding capacity of osteocalcin indicates a possible role in bone mineralization, but its exact function is still unclear. However, it is widely used as a serum marker for bone mineralization. Protein S, mainly a coagulant, is also vitamin-K dependent and synthesized in the liver. Children with

reduced protein S levels express enhanced blood clotting and a reduced bone density. MGP has been found in several supportive tissues (bone, cartilage) and in soft tissues including blood vessels. At least animal studies gave hints that MGP prevents soft tissues as well as cartilage from calcification while on the other hand it facilitates bone development and growth. Even the phenotype of mice lacking MGP supports this description, but the molecular mechanisms are still unknown. Rat studies with warfarin (vitamin K antagonist, see Fig. 1) led to prominent deformations of the skeleton of fetuses as well as newborns including excessive mineralization of growth plates and nasal cartilage, and stunted growth. Comparable effects were seen in humans after a therapy with vitamin K antagonists during pregnancy. The so called warfarin-embryopathy includes hypercalcification of the growth plates (Chondroplasia punctata), a nasal hypoplasia and disturbances in the growth of facial and hollow bones. For a more detailed overview see.

Cell Growth

Growth arrest-specific gene 6 (Gas6) is a γ -carboxylated protein found throughout the nervous system, in the heart, lung, stomach, kidneys, and cartilage. It is regarded as a ligand for several families of receptor tyrosine kinases (RTKs) which, when overexpressed, have effects on malignant cell survival. The exact mechanisms of Gas6 function have not yet been clarified but it is discussed as a cellular growth regulation factor with cell signaling activities as well as a supporter of

hematopoietic cells in the bone marrow. In addition, a role in the developing and the aging nervous system is under discussion and protective effects for cortical neurons have been shown. Other groups described an enhanced proportion of neurite-bearing PCD12 cells and acetylcholinesterase activity when cells were treated with nerve growth factor (NGF) and vitamin K in comparison to NGF treatment only which could be blocked by protein kinase A or MAP kinase inhibitors.

Clinical Use (Including Side Effects)

Vitamin K Deficiency

There are clearcut differences in deficiencies concerning infants, especially newborns, and adults. Deficiencies of vitamin K are really seldom in healthy adults, because the supply through daily food is (more than) enough, vitamin K is repeatedly recycled and therefore only a small loss appears, and – although not really accepted and because of the K2 production by intestinal bacteria (see above). On the other hand, those people with severe liver diseases or taking vitamin K antagonist anticoagulants have a risk of vitamin K deficiency. Symptoms are impaired blood clotting as revealed by routine tests measuring clotting time, unusual bleeding after smaller wounding, nose bleeding, heavy menstrual bleeding or a great susceptibility to hematoma.

The picture is different concerning infants during the first days of life, especially for those being breast fed. Coagulation-related (vitamin K-dependent) plasma proteins develop slowly during pregnancy resulting in markedly reduced levels after birth. In addition, human milk is a low-vitamin K nutrient, the newborns intestine almost totally lacks menaquinone-synthetizing bacteria, and the vitamin K cycle might not yet be established. These deficiencies can lead in healthy newborns to uncontrolled, in worst cases lethal intracranial bleedings. Due to that risk, in most countries newborns are supplied with phyloquinones, normally by injections, or by bottle feeding. Although there have been some hints in retrospective studies in the early 1990s on a correlation of vitamin K supplementation of newborns with the development of leucaemia, other retrospective studies found no correlations [2]. Therefore, a routine vitamin K prophylaxis of newborns is still recommended because the risk of early postnatal bleedings is much greater than the (unproven) risk of cancer.

Osteoporosis

This illness is mainly characterized by an age-related bone loss. The detection of osteocalcin in bone was the starting point for a series of studies on the role of vitamin K-dependent proteins in bone development and maintenance and on possible supplementantion therapies. Epidemiologic studies found differences in the risk of hip fractures depending on the dietary vitamin K

intake (35% risk at 250 µg/day of those with 50 µg/day), although no association between dietary intake and bone density could be found. Osteocalcin has been found to be a reliable marker of bone formation and vitamin K is necessary for the carboxylation of this protein. Therefore, the degree of carboxylation of osteocalcin is a reliable marker for the respective vitamin status. Blood levels of undercarboxylated osteocalcin were found to be higher in postmenopausal women and markedly higher in women over 70. Studies with elderly women resulted in a positive correlation of the blood level of undercarboxylated osteocalcin and the risk of bone fractures. Interestingly, also a correlation of undercarboxylated osteocalcin with the vitamin D nutritional status were described.

Application of vitamin K-antagonistic anticoagulants like warfarin gave no clearcut results concerning bone density or a changed risk for bone fractures.

Supplementation with high doses of vitamin K1 (1 mg/day for 14 days) or MK-4 (45 mg/day) resulted in decreased levels of undercarboxylated osteocalcin and increase of bone formation markers and in a significant reduction in bone loss, respectively. Using such high doses, any kind of effects besides vitamin K can not yet be ruled out and have to be further elucidated by long term studies. An overview can be found in a review by Palacios [4].

Atherosclerosis

In contrast to the formation and calcification of bones, vitamin K seems to lower the risk of aortic calcification. The mechanisms for these “antagonistic” effects is not known but a participation of osteocalcin (expressed in atherosclerotic plaques) as well as of matrix Gla protein (MGP) are discussed. In addition, the vitamin K epoxide reductase complex seems to be involved [5].

Safety and Toxicity

As the above mentioned studies with high supplementation dosages exemplarily show, there is no known toxicity for phyloquinone (vitamin K1), although allergic reactions are possible. This is NOT true for menadione (vitamin K3) that can interfere with glutathione, a natural antioxidant, resulting in oxidative stress and cell membrane damage. Injections of menadione in infants led to jaundice and hemolytic anemia and therefore should not be used for the treatment of vitamin K deficiency.

People using warfarin or other anticoagulants should try to consume the recommended daily intake very regularly to avoid interferences with their anticoagulant dosage adjusted by the physician. Interestingly, high doses of vitamins A and E seem to antagonize vitamin K, vitamin A interfering with vitamin K absorption and vitamin E (tocopherol quinone) inhibiting vitamin K-dependent carboxylase enzymes.

Vitamin K. Table 1 Vitamin K content of selected nutrients in alphabetic order

Nutrient	Vitamin K content ($\mu\text{g}/100\text{g}$)
Bovine meat	210
Broccoli	130
Brussels sprouts	570
Butter	60
Cauliflower	300
Chicken	300
Chicken egg	45
Mayonnaise	81
Milk (3.5% fat)	3.7
Oil (sunflower)	500
Porcine meat	18
Rice, white	1
Sauerkraut	1540
Soyabeans (semen)	190
Spinach	810
Tea, black leaves	262
Tea, green leaves	1428
Tomatoes	8
Wheat (germs)	350
Wheat (grains)	17

The adequate intake level (AI) for the US given by the FNB of the Institute of Medicine is 120 $\mu\text{g}/\text{day}$ and 90 $\mu\text{g}/\text{day}$ for men and women elder than 19 years, respectively. In Germany, 60–80 $\mu\text{g}/\text{day}$ for adults, 4 $\mu\text{g}/\text{day}$ for sucklings <4 months, and 50 $\mu\text{g}/\text{day}$ for children <15 years are proposed by the German nutrition society (DGE).

Food Sources

Phylloquinone (vitamin K1) is the form of vitamin K synthesized by mainly green leafy vegetables and such also appears in plant oils (soybean, cottonseed, canola, olive). Both are good sources for a daily supply, although the need of such a supply is still under discussion. Table 1 shows some good sources and their content of vitamin K1.

► Anticoagulants

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VLCS

► Fatty Acid Transporters

VLDL

► Very Low Density Lipoprotein

VMAT

VMAT is short for Vesicular Monoamine Transporter.

► Vesicular Transporters

Voltage-dependent Ca^{2+} Channels

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Synonyms

High voltage-activated (HVA) calcium channels; Low voltage-activated (LVA) calcium channels

Definition

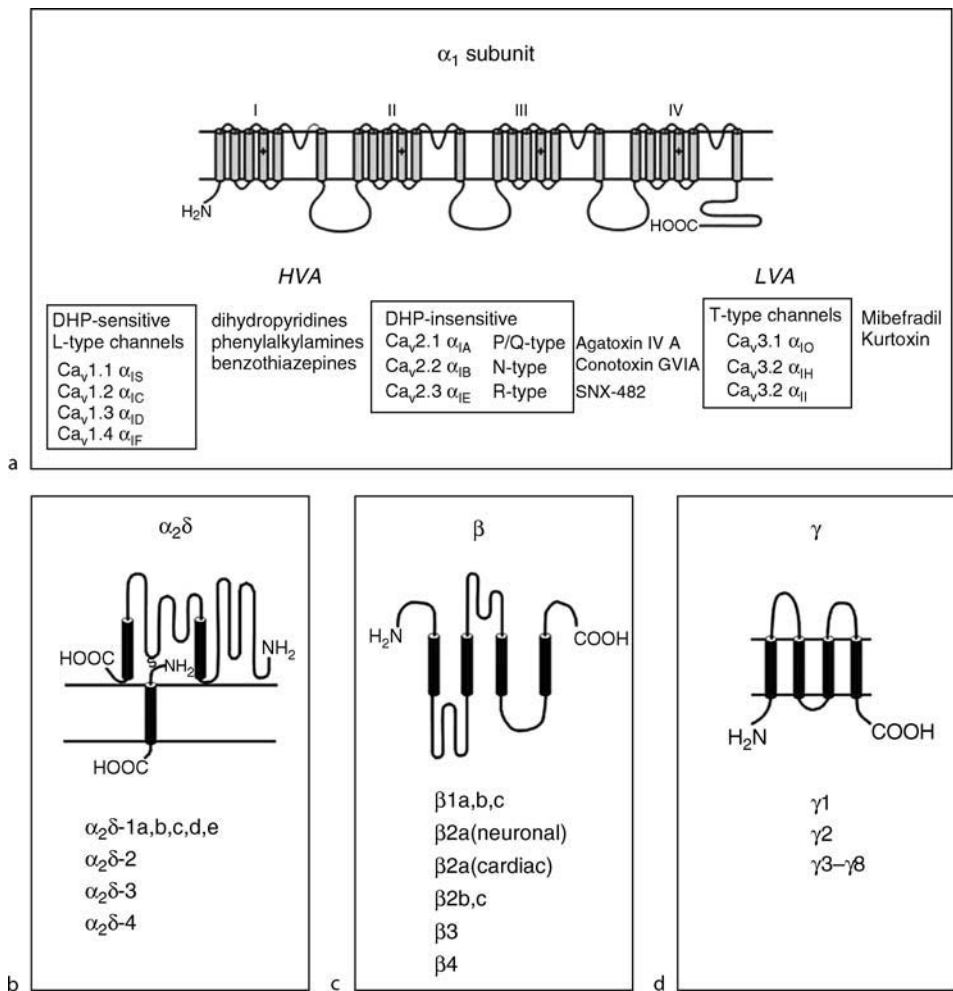
► Voltage-dependent Ca^{2+} channels are a family of multi-subunit complexes of five proteins responding to membrane depolarisation with channel opening allowing the influx of calcium into a cell. Voltage-dependent calcium channels are subdivided into three subfamilies: the HVA DHP-sensitive L-type calcium channels, the HVA DHP-insensitive calcium channels and the LVA T-type calcium channels [2].

Basic Characteristics

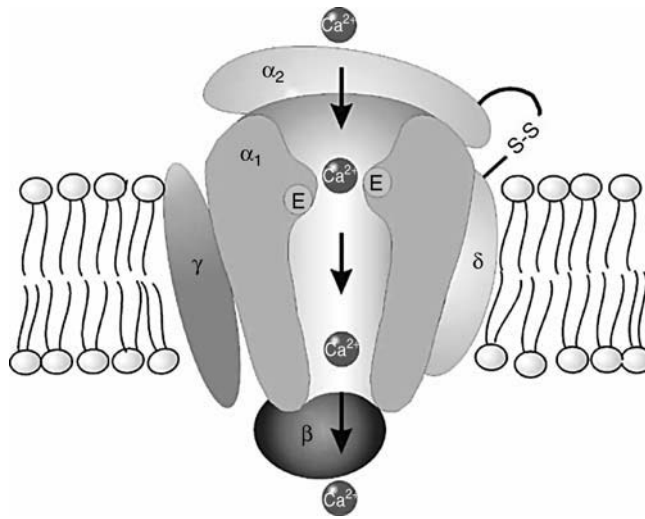
Voltage-activated calcium channels regulate the intracellular calcium concentration and contribute thereby to calcium signalling in numerous cell types. These channels are widely distributed in the animal kingdom and are an essential part of many excitable and non-excitable mammalian cell signalling pathways. Electrophysiological studies characterised different calcium currents identified as L-, N-, P-, Q-, R- and T-type current [1, 2]. The opening of these channels is primarily triggered by depolarisation of the membrane potential, but is also modulated by a wide variety of hormones, protein kinases, protein phosphatases, toxins and drugs. Site-directed mutagenesis has identified sites on these channels, which interact specifically with other proteins, inhibitors and ions.

HVA calcium channels are biochemically heterooligomeric complexes of five proteins encoded by four gene families (Fig. 1): The α_1 subunits of ~190–250 kDa contain the voltage-sensor, the selectivity filter, the ion-conducting pore, the binding sites for most calcium

channel blockers, and the interaction sites for ▶heterotrimeric GTP-binding protein subunits and other proteins. Seven α_1 genes have been identified (Fig. 1). Four genes ($\text{Ca}_v1.x$) encode L-type, dihydropyridine-sensitive channels. Three genes ($\text{Ca}_v2.x$) encode the none dihydropyridine-sensitive neuronal N-, P-, Q-, R-current. These α_1 subunits are associated with four auxiliary proteins (Fig. 2). The $\alpha_2\delta$ subunits, disulfide-linked dimers, are transcribed from four different genes and are clipped posttranslationally into the extracellular located α_2 - and the transmembrane δ -protein. The intracellular located β subunit and the transmembrane γ subunit are encoded by four and up to eight distinct genes, respectively. With the exception of the skeletal muscle calcium channel, a heterooligomer containing the $\text{Ca}_v1.1$ (α_{1S}), β_1 , $\alpha_2\delta$ -1 and γ_1 subunit, the exact composition of individual channels is not known. The identity of these complexes is further complicated by the existence of several splice variants for most genes that confer significant effects on the electrophysiological and/or pharmacological properties of the channels.



Voltage-dependent Ca^{2+} Channels. Figure 1 Structure, identity and blockers of calcium channel subunits.



Voltage-dependent Ca^{2+} Channels. Figure 2 Subunit composition of a HVA calcium channel. The selectivity filter of the channel is created by four glutamates (E).

The exact subunit composition of the **Low Voltage-activated (LVA) Ca^{2+} channels** is unknown [3, 6]. Three α_1 subunits, $\text{Ca}_v3.x$, have been identified which induce large T-type current after expression in *Xenopus* oocytes and in HEK cells in the absence of additional subunits. The T-type current can be affected by the $\alpha_2\delta$ and the γ_6 subunit suggesting a maximal subunit composition of $\alpha_1/\alpha_2\delta/\gamma$.

Properties of the α_1 Subunits: Each α_1 subunit contains four repeats that are composed of six transmembrane helices and a pore region between helix S5 and S6. The selectivity filter of the channels is located in the pore region and calcium selectivity of the HVA channels is created by four glutamates (E) in each pore, whereas the LVA channels have glutamates in the pore of repeat I and II and aspartates (D) in that of repeat III and IV. Conformational changes of the protein allow the channel to occupy one of three states: the closed, open or inactivated state. The change in membrane potential is sensed by the S4 helices of each repeat which contain a number of positive charged amino acids. Movement of these helices induces opening of the channel pore. HVA channels are activated approximately at a membrane potential of -30 mV with a maximal activation around 0 mV, whereas the LVA channels activate at potentials around -60 mV and have a maximal inward current around -10 mV. The LVA channels active and inactivate faster than the HVA channels. Inactivation of all channels is voltage-dependent and accelerated if the membrane is depolarised for prolonged time. Voltage-dependent inactivation may be mediated by sequences present in repeat I and II. Some of the HVA channels, especially the $\text{Ca}_v1.2$ channel, show calcium-dependent inactivation. Calcium flowing through the channel binds on the internal side to

calmodulin, a calcium binding protein tethered to the carboxy terminal tail of the α_1 protein by an isoleucine/glutamine (IQ)-motive. The calcium-calmodulin complex induces a conformational change in the α_1 protein that leads to inactivation of the channel. The $\text{Ca}_v1.4$ and the LVA channels do not have this type of inactivation.

The intracellular loop between repeat II and III couples and signals to intracellular effectors. The II–III loop of the $\text{Ca}_v1.1$ protein couples directly with the ryanodine receptor 1 located at the sarcoplasmic reticulum in the skeletal muscle triad. In contrast, the cardiac $\text{Ca}_v1.2$ channel is in close proximity but not in direct contact with the cardiac ryanodine receptor 2. This cardiac ryanodine receptor channel is activated by the calcium flowing through the open $\text{Ca}_v1.2$ channel. The II–III loops of the neuronal presynaptic localised $\text{Ca}_v2.1$ and $\text{Ca}_v2.2$ channels interact directly with the SNARE complex of the neurotransmitter containing vesicles and facilitates together with the inflowing calcium fusion of the vesicle and plasma membrane resulting in opening of the vesicle. Similar interactions occur probably between the $\text{Ca}_v1.2$ II–III loop and insulin containing vesicles of the pancreatic islets.

Neurotransmitter receptors inhibit the neuronal $\text{Ca}_v2.x$ channels by activation of G-proteins. The β/γ subunit of the **heterotrimeric GTP-binding proteins** binds to a QxxER sequence that is located at the I–II loop. Binding of the β/γ subunits confers the “reluctant phenotype” to these channels. This phenotype includes a reduced channel activation and current at normal depolarised membrane potentials. The I–II loop interacts also with the calcium channel β subunit with high affinity allowing transport of the α_1 protein from the Golgi to the membrane surface and a shift in the voltage-dependence of channel opening and closing.

► **Channel blockers:** Distinct blockers have been identified for the various HVA calcium channels which are listed in Fig. 1. ► **Dihydropyridines**, ► **phenylalkylamines** and ► **benzothiazepines** block all $\text{Ca}_v1.x$ L-type calcium channels. These compounds are used mainly to lower the blood pressure in hypertensive patients. In therapeutic concentrations, they block mainly the smooth muscle $\text{Ca}_v1.2$ channel. The dihydropyridines bind with high affinity to the inactivated state of the L-type channels. Binding requires amino acids on the IIS5, IIS6 and IVS6 helices. Mutation of Thr1061 in IIS5 to tyrosine abolished completely the high affinity interaction of dihydropyridines with the $\text{Ca}_v1.2$ channel protein. The difference in the sensitivity between the cardiac and the smooth muscle L-type calcium channel is in part caused by the use of alternative exons (No. 8) coding for the IS6 helix [7]. The phenylalkylamine and benzothiazepine binding sites contain amino acids present in the IIS6 and IVS6 helices and the glutamates in the pore region of repeat III and IV. The latter two groups include compounds that block neuronal $\text{Ca}_v2.x$ channels at similar concentrations as the $\text{Ca}_v1.x$ channels.

The LVA α_1 subunits are blocked by moderate to low (10 μM) concentrations of nickel and bind the channel blocker mibefradil and kurotoxin. Both compounds are not specific LVA channel blockers because they block also $\text{Ca}_v1.x$ and $\text{Ca}_v2.x$ channels at about tenfold higher concentration. Interestingly, the endogenous cannabinoid ► **anandamide** binds to LVA channels and stabilises the inactivated state. This effect decreases T-type calcium current and neuronal firing activities.

The $\alpha_2\delta$ subunit 1 and 2 bind gabapentin with high affinity. This interaction may be causally related to its antiepileptic and neuropathic pain alleviating property.

Localisation of channels: The $\text{Ca}_v1.1$ channel is present in skeletal muscle at the triad. The $\text{Ca}_v1.2$ channel is widely distributed and represents the major L-type calcium channel in most tissues. In contrast, the $\text{Ca}_v1.4$ channel has been detected only in the retina, so far. The $\text{Ca}_v1.3$ (α_{1D}) channel is mainly found in neuroendocrine cells and the inner ear. The $\text{Ca}_v2.1$, $\text{Ca}_v2.2$ and $\text{Ca}_v2.3$ are mainly localised presynaptic. The $\text{Ca}_v2.1$ and $\text{Ca}_v2.2$ channels interact with the vesicular release machinery and regulate the release of neurotransmitters.

The LVA channels are expressed in a wide variety of tissues. In the cardiac sinus node and the thalamus, activation of LVA channels seems to be necessary to generate action potentials upon depolarising the membrane.

Mutation and Deletion

Deletion of the $\text{Ca}_v1.1$ and $\text{Ca}_v1.2$ gene is not compatible with viable mouse pups [4, 5]. The $\text{Ca}_v1.2$ channel is absolutely required for the contraction of the developing mouse heart after embryonal day 14.

Mutation of the human $\text{Ca}_v1.1$ gene is associated with hypokalemic periodic paralysis. Deletion of the $\text{Ca}_v1.3$ gene leads to viable pups that are deaf and have cardiac arrhythmia at rest. Mutation of the human $\text{Ca}_v1.4$ gene is associated with X-linked congenital stationary night blindness.

Mutation in the neuronal $\text{Ca}_v2.1$ channel is associated with familial hemiplegic migraine and episodic ataxia in humans. Deletion of the $\text{Ca}_v2.1$, $\text{Ca}_v2.2$ and $\text{Ca}_v2.3$ gene is compatible with life accompanied by a variety of central and peripheral defects.

Deletion of the $\text{Ca}_v3.1$ channel in thalamocortical relay neurons prevents ► **absence epilepsy**. Block of the neuronal LVA channels alleviates certain forms of epilepsy. Deletion of $\text{Ca}_v3.2$ leads to coronary artery constriction and focal myocardial fibrosis.

Deletion of the $\beta 1$ subunit is lethal, whereas deletion or mutation of the $\alpha_2\delta$ -2, $\beta 3$, $\beta 4$, $\gamma 1$ and $\gamma 2$ genes is associated either with no or various neuronal phenotypes.

More recent analysis of tissue specific gene deletions showed that the $\text{Ca}_v1.2$ channel is involved in a wide variety of function including hippocampal learning, insulin secretion, intestine and bladder motility. Further analysis will be required to unravel the functional significance of voltage-dependent calcium channels for specific cellular functions.

Drugs

Numerous dihydropyridine calcium channel blockers have been introduced to treat hypertension and stable angina pectoris. Nifedipine, nitrendipine, nisoldipine, nilvadipine, nicardipine, amlodipine, felodipine, isradipine block preferentially the vascular, smooth muscle $\text{Ca}_v1.2$ calcium channel at therapeutic doses. Nimodipine that has a short half life has been used to alleviate cerebral vasospasms after subarachnoidal bleeding. The phenylalkylamines verapamil and gallopamil and the benzothiazepine diltiazem have been used as antihypertensive drugs and to treat supraventricular tachyarrhythmia. Mibefradil – a compound thought to inhibit preferentially T-type channels – that blocks at low concentrations vascular $\text{Ca}_v1.2$ L-type channels in the mouse, has been used for a short period as an antihypertensive drug. It has been removed from the market due to intolerable interactions with other drugs.

Gabapentin and pregabalin are prescribed in certain epileptic diseases such as absence epilepsy and in neuropathic pain. Their therapeutic target for pain suppression is the $\alpha_2\delta$ -1 subunit.

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Voltage-dependent Inactivation

Voltage-dependent inactivation is channel inactivation at depolarised membrane potentials.

► Voltage-dependent Ca²⁺ Channels

Voltage-dependent Na⁺ Channels

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Synonyms

Voltage-gated sodium channel; Na⁺ channel

Definition

Voltage-dependent sodium channels are a family of membrane proteins that mediate rapid Na⁺ influx, in response to membrane depolarization to generate action potentials in excitable cells.

Basic Characteristics

Electrophysiological studies (mainly using voltage-clamp and patch clamp) revealed the essential properties of the sodium channels; kinetics of channel ►gating and selective ion permeation. Sodium channels are

closed at negative resting membrane potentials. Membrane depolarization evokes very rapid activation of the channel, followed by inactivation typically within several milliseconds. The sodium channels recover quickly from fast inactivation when the membrane is repolarized. When the membrane is depolarized for a longer period (from hundreds of milliseconds to seconds), the sodium channels undergo slow inactivation. There are probably multiple types of slow inactivation. Recovery from slow inactivation requires a longer time of repolarization.

The sodium channels are very selective for Na⁺ over K⁺, allowing Na⁺ influx down the electrochemical gradient to generate positive membrane potentials. The sodium channels are also permeable to Li⁺ and NH₄⁺. The narrowest portion of the channel pore is estimated to be rectangular (3.1 × 5.2 Å).

In addition to the ionic current, membrane depolarization evokes the gating current even in the absence of permeant ions. This tiny current represents the movements of charged parts of the sodium channel proteins. Comparison of the voltage-dependence of ionic currents and gating currents, together with other lines of evidence, suggests that the sodium channels undergo multiple conformational transitions between the resting and open states. These transitions are successfully simulated using a framework of Markov process. Conformational changes are essential for state-dependent effects of various drugs acting on the sodium channels.

The primary role of the sodium channels is to generate action potentials in excitable cells. In case of neurons, the sodium channel density is high at axon hillocks or axon initial segment where action potentials start to propagate. The sodium channels are also present in dendrites. The sodium channels contribute to amplifying synaptic inputs (particularly those distally located) and are actively involved in ►back propagation of action potentials into dendrites. Subtle differences in properties of sodium channels influence the dendritic processes of synaptic integration in complex ways.

Molecular Structure

The three-dimensional structure of the sodium channel (from electric eel) was determined at 19-Å resolution using cryo-electron microscopy and single-particle image analysis. The sodium channel has a bell-shaped outer surface of 135 Å in height, 100 Å in side length at the square bottom, and 65 Å in diameter of the spherical top. An interesting finding is that there are several inner cavities connected to outer orifices.

Molecularly, mammalian voltage-dependent sodium channels are composed of the main pore forming α subunit and smaller auxiliary β subunits. The rat brain sodium channel contains the 260-kD α subunit, the 36-kD β1 subunit and the 33-kD β2 subunit. The subunit stoichiometry is α: β1: β2 = 1: 1: 1.

The α subunit is the main component of the sodium channels, consisting of $\sim 2,000$ amino acid residues. Amino acid sequence analysis reveals four repeated units of homology (repeat I–repeat IV), each containing six hydrophobic, putative transmembrane segments. The N- and C-termini and the linking regions between repeats are assumed to reside in the cytoplasmic side. The fourth hydrophobic segment, S4, of each repeat has a well-conserved motif of positively charged residues appearing every third residues. This motif is found in other voltage-dependent channels, and contributes to sensing voltage changes. S4 moves outward in response to depolarization and becomes accessible from the extracellular side. Among sodium channel subtypes, the linker regions between repeats are less homologous than the transmembrane regions, except for the linker connecting repeats III and IV.

The conserved linker between repeats III and IV is critical for fast inactivation. Cleavage of the III–IV linkage causes a strong reduction in the rate of inactivation. A cluster of three hydrophobic residues (IFM) in the linker is an essential component, probably serving as a hydrophobic latch to stabilize the inactivated state. Other parts of the α subunit are also involved in fast inactivation. Conformational changes in the P region contribute to the slow inactivation process.

The region between S5 and S6 of each repeat is commonly called “P region” (P for “pore”), and is important for forming the narrow part of the ion conducting pathway and the selectivity filter. Mutagenesis studies identified the most critical amino acid residues, D, E, K, and A for repeats I–IV, respectively. Interestingly, alteration of K to E in repeat III and A to E in repeat IV dramatically change the ion-selectivity properties, to resemble those of calcium channels, suggesting that these amino acid residues participate in forming the selectivity filter. This observation also suggests a close evolutionary relationship between the voltage-dependent sodium and calcium channels, although a prokaryotic voltage-gated sodium channel is encoded by a single domain. The “P region” also forms the binding site for tetrodotoxin

▶(TTX) and ▶saxitoxin, which block the channel pore from the outer side. The difference in ▶TTX sensitivity among the sodium channels is caused by a single amino acid difference in the “P region” of repeat I (phenylalanine or tyrosine in TTX-sensitive channels; cysteine or serine in TTX-resistant channels). The S6 segments contribute to forming the inner pore of the channel and binding sites for local anesthetics.

Molecular cloning has detected multiple α subunit genes, more than expected from electrophysiological and pharmacological measurements (Table 1). The primary transcript from a sodium channel gene undergoes a developmentally regulated complex pattern of alternative splicing, generating further heterogeneity. The sodium channels are practically classified into two major classes based on the sensitivity to ▶TTX. The sodium channels are relatively similar in properties, showing rapid activation and inactivation.

Note that the numbering of the new system is not the same as for the gene symbols. A closely related sodium channel-like proteins have been identified and named Na_x. Functional properties of these proteins are not known, except for the NaG protein, which functions as a sodium sensor of body fluid.

The β subunits ($\beta 1$ – $\beta 4$) are membrane proteins with a single transmembrane domain and an extracellular immunoglobulin-like motif, and perform the regulatory roles of the sodium channel. The $\beta 1$ subunit accelerates the activation and inactivation kinetics. The $\beta 2$ subunit is covalently linked to the α subunit, and is necessary for the efficient assembly of the channel. The more recently identified $\beta 3$ subunit is homologous to $\beta 1$, but differs in its distribution within the brain and in a weaker accelerating property. The $\beta 4$ subunit is similar to $\beta 2$ and is covalently linked to the α subunit.

Functions of and Associated Disorders of Sodium Channel Subtypes

CNS neurons express at least four types of sodium channels, Na_v1.1, Na_v1.2, Na_v1.3, and Na_v1.6. Na_v1.1 is found in the somatodendritic membrane,

Voltage-dependent Na⁺ Channels. Table 1 Mammalian sodium channel α subunits

Type	Conventional names	Gene symbol (human)	TTX sensitivity	Primary tissues
Na _v 1.1	Brain type I	SCN1A	S	CNS, PNS
Na _v 1.2	Brain type II, type IIA	SCN2A	S	CNS
Na _v 1.3	Brain type III	SCN3A	S	CNS, PNS
Na _v 1.4	$\mu 1$, SkM1, SCN4A	SCN4A	S	skeletal muscle
Na _v 1.5	Heart 1, SkM2, hH1, SCN5A	SCN5A	R	heart
Na _v 1.6	NaCh6, Scna8, PN4, CerIII	SCN8A	S	CNS, PNS
Na _v 1.7	hNE-Na, Na _s , PN1, Scn9a	SCN9A	S	PNS
Na _v 1.8	SNS, PN3, NaNG, Scn10a	SCN10A	R	PNS
Na _v 1.9	NaN, SNS2	SCN11A	R	PNS
Na _x	Na _v 2.1, Na _v 2.3, NaG, SCL-11	SCN7A		CNS, PNS, heart, uterus

whereas Na_V1.2 is predominantly found on axons and at or near axon terminals. A sequence of nine amino acid residues has been identified in the cytoplasmic C-terminus of Na_V1.2, which mediates axonal compartmentalization. Na_V1.2 is likely to be involved in action potential initiation at the initial segment. Knock-out of Na_V1.2 is lethal. Na_V1.3 is expressed predominantly in embryonic and early postnatal stages. Na_V1.6 is localized at Nodes of Ranvier and dendrites (Fig. 1).

In cerebellar Purkinje cells, a TTX-sensitive inward current is elicited, when the membrane was partially repolarized after strong depolarization. This “resurgent current” contributes to high-frequency repetitive firing of Purkinje neurons. The resurgent current results from open channel block by the cytoplasmic tail of the β4 subunit. The *med* Na_V1.6 mutant mice show defective synaptic transmission in the neuromuscular junction and degeneration of cerebellar Purkinje cells.

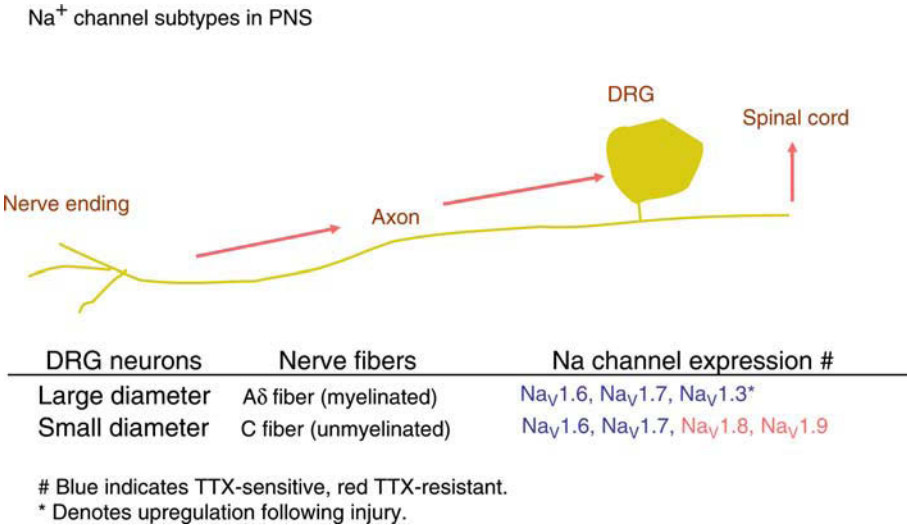
Generalized epilepsy with febrile seizures plus (GEFS+) is an autosomal dominantly inherited syndrome, which is distinct from febrile seizures, displaying seizures persisting beyond 6 years of age and generalized epilepsies. GEFS + is associated with mutations in the genes encoding the sodium channel β1 subunit (GEFS + type 1), the Na_V1.1 α subunit (GEFS + type 2), and the Na_V1.2 α subunit. GEFS + also results from GABA_A receptor dysfunction. Severe myoclonic epilepsy of infancy is associated with Na_V1.1 mutations.

In peripheral nervous system, Na_V1.7, Na_V1.8, and Na_V1.9 are expressed in addition to the CNS sodium channel subtypes. Whereas large-diameter neurons in dorsal root ganglia (DRG) generate TTX-sensitive currents (presumably mediated by Na_V1.6 and Na_V1.7), small cells also generate TTX-resistant currents (presumably mediated by Na_V1.8, possibly by Na_V1.9).

The Na_V1.7 has slow closed-state inactivation and carries a significant current in response to ramp depolarization. The results suggest that Na_V1.7 amplifies the “generator potentials” in nociceptor nerve endings. Recently, clinical studies revealed a critical role of Na_V1.7 for nociception. Missense mutations were identified in patients with inherited primary erythromelalgia, a disorder characterized by intermittent pain, redness, heat, and swelling in the extremities. Furthermore, genetic analysis of patients with “congenital indifference to pain” showed that three nonsense mutations abolished the Na_V1.7 channel function. When peripheral nerves are damaged, expression of Na_V1.8 and Na_V1.9 are downregulated, whereas Na_V1.3 is upregulated. The β3 subunit is also upregulated in injured nerves. This deranged sodium channel expression may cause aberrant ectopic activity in sensory neurons, generating ▶neuropathic pains.

Mutations of the Na_V1.4 channel gene cause various types of muscle diseases, including hyperkalemic periodic paralysis, paramyotonia congenita, ▶myotonia fluctuans, acetazolamide-sensitive myotonia. Mutations disrupt inactivation and cause both myotonia (enhanced excitability) and attacks of paralysis (inexcitability resulting from depolarization).

In the ▶Long QT Syndrome (LQTS), the repolarization phase of the cardiac muscle is delayed, rendering the heart vulnerable to an “arrhythmia” known as torsade de pointes. LQTS is associated with five genes encoding ion channels. LQTS type 3 (LQT3) results from mutations of Na_V1.5, which cause persistent sodium current. In contrast, sodium channel mutations associated with Brugada syndrome reduce the expression level of cardiac sodium channels.



Voltage-dependent Na⁺ Channels. Figure 1 Na⁺ Channel subtypes in PNS.

Channel Modulation

Protein kinase A attenuates sodium current amplitude by phosphorylating serines located in the I–II linker. There is a consensus protein kinase C phosphorylation site in the III–IV linker. Activation of protein kinase C decreases peak sodium current and slows its inactivation. Sodium channels interact with G proteins. Coexpression of G protein $\beta\gamma$ subunits greatly enhances sodium currents, slows inactivation, and shifts the steady state inactivation curve to the depolarizing direction. The C-terminal region of the sodium channel contains the G $\beta\gamma$ -binding motif Q-X-X-E-R, suggesting that the sodium channel is directly modulated by G $\beta\gamma$ subunits. The sodium channel has the IQ-motif sequence of non-Ca²⁺-dependent calmodulin binding in the C-terminal region. This motif is conserved in various types of sodium channel, but its physiological function remains elucidated. The sodium channel interacts with a variety of proteins, which include AKAP15 (15 kDa A-kinase anchoring protein), ankyrin-G, 14-3-3, dystrophin, and syntrophin.

Drugs

The sodium channels are targets of various chemicals and drugs. TTX and saxitoxin block the channel pore from the outside. Lidocaine and other local anesthetics act on the S6 transmembrane segment of repeat IV (IVS6), and delay the recovery from the inactivated state. Batrachotoxin, aconitine, and grayanotoxin interact with IS6, to shift the voltage-dependent activation and to slow inactivation. Scorpion toxins slow inactivation (α -toxins) and shift the voltage dependence of activation to more negative potentials (β -toxins). The peptide toxin μ -conotoxin GIIIB is a specific blocker for Na_v1.4, but no other pharmacological tools are available to specifically discriminate the sodium channel subtypes.

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Voltage-gated K⁺ Channels

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Synonyms

Kv-channels

Definition

Voltage-gated potassium (Kv) channels are membrane-inserted protein complexes, which form potassium-selective pores that are gated by changes in the potential across the membrane. The potassium current flow through the open channel follows by the electrochemical gradient as defined by the Nernst equation. In general, Kv channels are localized in the plasma membrane.

Basic Characteristics

Functional Characteristics

Kv channels may be important determinants of cellular activities correlated with changes in membrane potential. Examples range from neural signal transduction, action potential wave forms, action potential propagation, action potential frequency, pacemaking and secretion to the regulation of cell volume and cell proliferation. In addition to changes in voltage, Kv-channel activities may be regulated by various physical and/or chemical stimuli. They include Na⁺, Ca²⁺, Mg²⁺, ATP, O₂, pH, pressure, redox potential, phosphorylation/dephosphorylation, G protein binding, interaction with cytoskeletal proteins, and more [1]. Once Kv channels have been activated, they catalyze a rapid passage of K⁺ through the open pore along the electrochemical gradient as defined by the Nernst equation. Activated Kv-channels often tend to inactivate [2, 3]. The kinetics of inactivation may occur in time ranges of ms to tens of seconds. The inactivation mechanism of ►Shaker-channels, which inactivate rapidly, has been thoroughly investigated. One mechanism utilizes an amino-terminal inactivating domain. This domain is able to bind to the open pore of Shaker-channels. Thereby, the pore becomes both occluded and locked in an open state. Upon repolarization, inactivated Kv-channels recover from inactivation. Inactivated Kv-channels are refractory to activation. In most circumstances, intracellular K⁺ concentrations are higher than the extracellular ones, and the membrane potential is positive to the Nernst potential. Therefore, the direction of K⁺ current flow through Kv-channels is

mostly outward. But there are important exceptions, where either the membrane potential at which the Kv-channel opens is negative to the Nernst potential or the extracellular K⁺ concentration is not very different from the intracellular one. For example, inactivated Kv-channels like ►**HERG-channels** (see above) may recover from inactivation at very negative membrane potentials. During recovery they pass through an open state permitting an inward flow of K⁺ current. Depending on the particular conditions, hyperpolarizing Kv-channel activity may attenuate cellular excitability (e.g., the firing of action potentials) or they may balance depolarizing activities (e.g., clamp the membrane potential to a certain value to allow a steady inward flow of calcium ions). Frequently, repolarizing Kv-channel activity shifts the membrane potential into a hyperpolarizing direction.

Kv-channels are closed in the resting state. Upon depolarization of the cellular membrane potential, closed Kv-channels undergo a series of voltage-dependent activating steps until they reach an activated state from which they can open and close in a voltage-independent manner.

A particularly interesting example of Kv-channel inactivation is represented by HERG-channels. HERG-channels have faster inactivation than activation kinetics, and they very rapidly recover from inactivation at negative membrane potentials. This behavior may result in a situation where most of the current carried by HERG-channels occurs during their recovery from inactivation at negative potentials, that is, it represents an inward rather than outward current.

Structural Characteristics of Kv-Channels

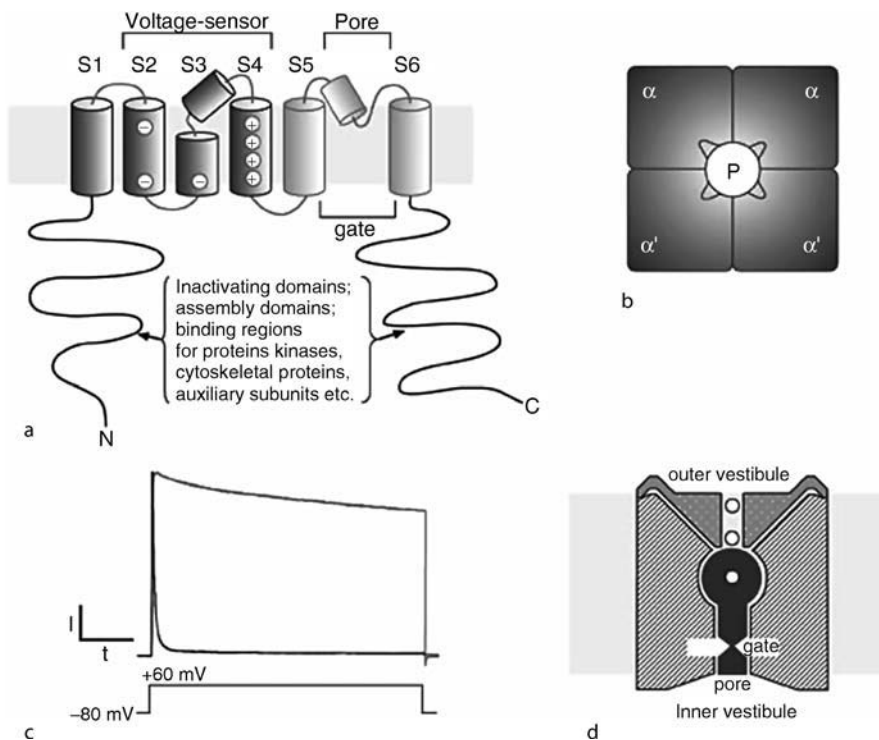
Kv-channels are frequently heteromultimeric protein assemblies of pore-forming membrane-integrated α -subunits and of auxiliary subunits [2, 3]. The first Kv-channel subunits were cloned from *Drosophila*. This work initiated the subsequent identification and cloning of many more Kv-channel genes constituting a superfamily of related proteins. The design of the proteins is structurally and functionally highly conserved. Kv-channel α -subunits have cytoplasmic amino- and carboxy-termini, which frame a membrane-spanning core domain. The core domain consists of six hydrophobic membrane-spanning segments S1 to S6. Segments S5 and S6 are linked by the so-called P-loop. This P-loop enters and exits the plasma membrane from the extracellular face. Figure 1a shows a cartoon of the most likely membrane topology of Kv-channel α -subunits. Four subunits are necessary to form a functional channel. Homo- as well as heterotetrameric assembly of Kv-channels is possible (Fig. 1b). Kv-channels can be expressed in vitro in heterologous expression systems (Fig. 1c). The relative ease to

in vitro mutagenise Kv-channel cDNAs and to express Kv-channel cDNAs heterologously in the *Xenopus* oocyte or tissue culture expression systems has produced a detailed understanding of many basic features concerning Kv-channel activity. The results showed that the voltage-sensing apparatus of Kv-channels is mainly formed by amino acids residing in segments S2 to S4. Most notably is the occurrence of a repeat sequence (R/KXX)_{n=3–5} in segment S4, lining up several positive charges in the membrane electric field. The charges apparently move in the electric field when the Kv-channels become activated, giving rise to a gating current across the membrane. Gating currents are observed during the voltage-dependent activation of Kv-channels.

Amino acid residues residing in the S5–P–S6 region are engaged in forming the pore, most notably a highly conserved P-loop sequence TVGY/FGD/N, which has been dubbed the K-channel signature sequence. This sequence forms part of the selectivity filter of the Kv-channel pore. It has been possible to crystallize a bacterial K-channel (KcsA). The crystals provide a high-resolution picture of the pore of a K-channel with its surrounding transmembrane helices [4]. The preservation of K-channel pore structures during evolution suggests that Kv-channels have a pore structure similar to the one of the KcsA-channel.

According to the KcsA crystal structure data, hallmarks of the pore structure are an outer vestibule with a relatively flat surface. Beneath, in the upper-third of the membrane, lies a narrow selectivity filter. This is followed by a central aqueous cavity narrowing into the internal mouth of the pore (Fig. 1d). Kv-channels, during voltage-dependent activation and opening, undergo marked conformational changes. In the resting state, it is assumed that the pore is closed by a “gate” and that upon activation the gate opens. Amino acid residues in the carboxy-terminal half of the S6 region seem to play a pivotal role for the structure and function of the Kv-channel gate. Other Kv channel domains e.g. the S4-S5 linker region, may also contribute to channel gating, likely by indirect and/or allosteric mechanisms.

In addition to the membrane-inserted core domain of Kv channels, their cytoplasmic domains have important roles for Kv-channel function [5]. Many of these functions are related to subunits assembly, channel trafficking to and from the plasma membrane, and interactions with cytoskeletal components (Fig. 1a). A tetramerization (T) domain for subunit assembly has been well defined in Shaker-channels, where it is localized in the amino-terminus. Other Kv-channels (e.g., eag, HERG, ►**KvLQT1**) may have comparable domains within the cytoplasmic carboxy-terminus. ER retention and retrieval signals have been found



Voltage-gated K⁺ Channels. Figure 1 Basic features of voltage-gated potassium channels. (a) Schematic drawing of the membrane topology of Kvα-subunits. Transmembrane segments are numbered S1–S6. The linker regions between segments S3/S4 and S5/S6 contain small α-helical regions marked as cylinders. Negative charges (–) in segments S2 and S3 and positive charges (+) in segment S4 contribute to the voltage-sensor of Kv-channels. Pore-forming residues are located within the S5/S6 linker region and segment S6. The gate which opens and closes Kv-channels is not exactly known. Amino acid residues of the S4/S5 linker region, segment S5, and segment S6 are directly and/or indirectly involved in the gating machinery. Brackets give examples for additional functions and properties associated with sequences and domains of the cytoplasmic amino- and/or carboxy-termini. (b) Assembly of four Kvα-subunits is needed to form functional Kv-channels with a central pore P. α and α' indicate that assembly of homo- and heteromultimers is possible. (c) Typical examples of potassium outward currents (I) mediated by Kv-channels upon jumping from a holding potential of –80 mV to a test potential of +60 mV. Black trace: rapidly inactivating outward current; gray trace: noninactivating delayed-rectifier type current. Time-scale (t) is in ms. Upon repolarization from a depolarizing test potential to a hyperpolarizing holding potential an inward current or tail current can be observed that reflects the closure of open channels. (d) Schematic diagram of pore structure. A hypothetical sagittal section through the pore is shown. The pore has an outer vestibule, a selectivity filter (dotted gray) in the upper third of the membrane, an aqueous cavity, a gate (black), and an inner water-filled vestibule. Potassium ions are drawn approximately to scale as white circles.

in the cytoplasmic amino- or carboxy-termini. Also, cytoplasmic Kv-channel domains may contain recognition sequences for a variety of serin/threonine and/or tyrosine kinases. Finally, motifs have been characterized that interact with a variety of cytoskeletal components. For example, the conserved carboxy-terminal amino-acid motif TDV is recognized by MAGUK(PSD)-proteins of the post- and presynaptic densities.

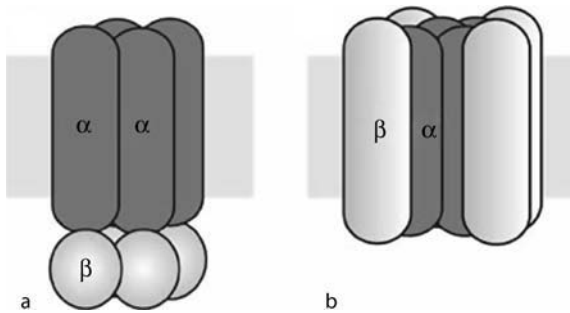
Auxiliary Subunits

Kvα-subunits may coassemble with auxiliary subunits (see Table 1). Auxiliary subunits (e.g., Kvβ-subunits)

may bind to cytoplasmic regions of the Kvα-subunits extending the reach of the membrane-integrated core channel into the cytoplasm (Fig. 2a). A particularly interesting group is the KChIP-family. KChIP stands for K-channel interacting protein. The proteins are small Ca²⁺-binding proteins being related to the superfamily of neuronal calcium sensors. They are tightly associated with somato-dendritic rapidly inactivating Kv-channels (Kv4.1, Kv4.2, Kv4.3). Alternatively, the auxiliary subunits may be membrane integrated proteins like Kvα-subunits (Fig. 2b). Examples are members of the KCNE-family of auxiliary subunits that may assemble with a great variety of Kv-channels including

Voltage-gated K⁺ Channels. Table 1 Auxiliary subunits and their reported interactions with Kv α -subunits

Auxiliary subunit	Kv α -subunits
Kv β 1, Kv β 2, Kv β 3	Kv1-family, Shaker
MIRP2	Kv3.4
KChIPs (KChIP1,2,3,4)	Kv4.1, Kv4.2, Kv4.3
KCNE1–KCNE4	KCNQ1–KCNQ5 HERG, Kv3.4, Kv4.3
slo β 1–slo β 4	BK(slo) α -subunits



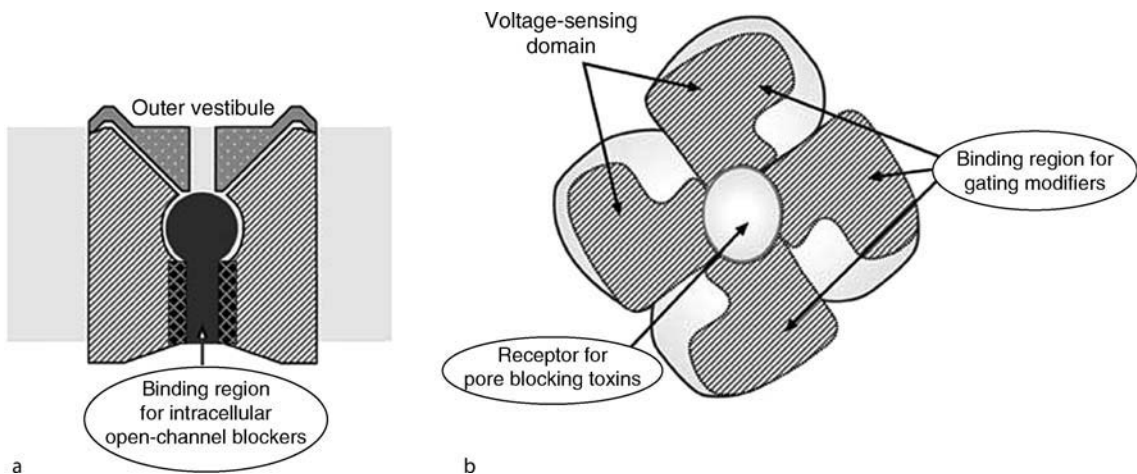
Voltage-gated K⁺ Channels. Figure 2 Schematic drawing of voltage-gated potassium channels as heteromultimeric assemblies of pore-forming Kv α -subunits and auxiliary subunits. (a) Assembly of Shaker-type Kv-channels with cytoplasmic subunits, for example, Kv β -subunits and KChIPs; (b) Assembly of Kv-channels with membrane-integrated auxiliary subunits, for example, MinK and MirPs. Membrane is shaded gray.

►KCNQ-channels, HERG-channels, and Shaker-related Kv-channels.

Functional activities of auxiliary subunits may include chaperone activities during assembly, for example, Kv β -subunits have been shown to exert a chaperone function for the assembly of Shaker α -subunits or the recognition of ER-retention signals as discussed for KChIPs. Notably, auxiliary subunits may modulate the biophysical and pharmacological properties of Kv-channels. Certain Kv β (Kv β 1.1 and Kv β 3.1) confer a rapid-inactivation behaviour to otherwise noninactivating Kv-channels. Association of KCNE1 with KvLQT1 leads to a significant slowing of Kv-channel activation, a depolarizing shift in the voltage dependence of activation and a change in drug sensitivity (e.g., for mefenamide). On the other hand, association of KCNE3 with KvLQT1 leads to channels that are no longer activated by voltage, but behave like potassium-selective pores in the membrane.

Drugs

In the animal kingdom, a great number of toxins from snakes to scorpions, insects, spiders, and sea anemones that block various Kv-channels with nano- to picomolar affinities have been identified. Two kinds of block may be discerned in general. The toxin may bind to the outer vestibule of the Kv-channel pore and thereby occlude the pore (Fig. 3a). In this case, the binding of one toxin per channel may suffice to block channel activity. Alternatively, the toxin may interfere with the voltage-sensing machinery and modify Kv-channel gating. The voltage-sensing machinery is located in the periphery of the pore. As each subunit appears to have its own



Voltage-gated K⁺ Channels. Figure 3 Schematic drawing of Kv-channel binding sites for toxins and drugs. (a) Side view of a cut-open Kv-channel. Intracellular open-channel blockers may bind to the inner vestibule of Kv-channels (hatched) formed by segment S6. (b) View of a Kv-channel from the top. Pore-blocking toxins bind to receptor sites formed by the Kv-channel pore and the outer vestibule. Gating modifiers have binding sites outside of the pore (striped) and interfere with the voltage-sensing machinery.

Voltage-gated K⁺ Channels. Table 2 Kv-channel genes associated with heritable diseases

Gene	Trivial name	Kv-channel type	Disease
KCNA 1	Kv1.1	Shaker-channel	EA
KCNH 2	HERG	IKr-channel	LQT
KCNE 1	MinK	Auxiliary subunit	LQT
KCNE 2	MirP1	Auxiliary subunit	LQT
KCNE 4	MirP2	Auxiliary subunit	Myopathy
KCNQ 1	KvLQT1	Delayed rectifier	LQT/JLN
KCNQ 2	–	M-channel	BFNC
KCNQ 3	–	M-channel	BFNC
KCNQ 4	–	M-channel	DFNB2

EA, episodic ataxia; LQT, long QT syndrome; JLN, Jervell–Lange Nielsen syndrome; BFNC, benign familial epilepsy; DFNB2, deafness syndrome

voltage-sensing apparatus, gating-modifying toxins may have four binding sites per Kv-channel (Fig. 3b). Characteristically, gating-modifying toxins induce a positive shift in the current–voltage relationship of Kv-channels and accelerate channel deactivation (closing of the open channel).

Many nonpeptidergic compounds are known to block Kv-channels. Where the mechanism of block is known, it is related to pore occlusion. Most frequently, nonpeptidergic drugs block the pore by interacting with amino acid residues located in the carboxy-terminal part of segment S6 (Fig. 3a). This part is particularly hydrophobic in the pore of HERG-channels. Apparently, it distinguishes HERG-channels from other Kv-channels and renders HERG-channels exceptionally sensitive to block by many pharmaceuticals. Since HERG-channels make an important contribution to cardiac action potential repolarization, HERG-channel block may be frequently responsible for cardiac side effects of drugs. Tetraethylammonium, 4-aminopyridine, and quinidine are unspecific drugs blocking Kv-channels by binding to the inner entrance of the pore. TEA may also bind to the outer entrance of Kv-channel pores.

Mutations in Human Kv-Genes Associated with Hereditary Channelopathies

Mutations in human Kv-channel genes have been detected that are associated with hereditary diseases ranging from heart arrhythmia (long QT-syndrome) and deafness to epilepsy and ataxia (see Table 2). Typically, many Kv-channel related channelopathies are correlated with a mutant phenotype that is episodic in nature and appears as a dominant hereditary trait.

Outlook

Several additional crystal structures of K⁺ channels have been solved since this article was written. Importantly,

crystal structure for the voltage-gated Kv1.2 channel in complexes with Kvβ2 has been solved (Long SB, Campbell EB, MacKinnon R (2005) Science 309, 897–903; see also: Long SB, Tao X, Campbell EB, MacKinnon R (2007) Nature 450, 376–382)

- **Antiarrhythmic Drugs**
- **ATP-dependent K⁺ Channel**
- **Inward Rectifier K⁺ Channels**
- **K⁺ Channels**

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Voltage-gated Na⁺ Channel

- **Voltage-dependent Na⁺ Channels**

Voltage Sensor

The voltage sensor is the part of a channel protein responsible for detection of the membrane potential. A voltage sensor of the voltage-dependent Na^+ channel was predicted by Hodgkin and Huxley in 1952. Positively charged amino acid residues in S4 of each repeat play an essential role as the voltage sensor.

► Voltage-dependent Na^+ Channels

von Willebrand Factor

The von Willebrand factor (vWf) is a heterogeneous multimeric plasma glycoprotein produced by megakaryocytes and endothelial cells which is found in platelets, plasma and the subendothelium. Subendothelial vWf facilitates platelet adhesion, especially under high shear stress, by binding to glycoprotein GPIb-V-IX, a complex of four leucine-rich repeat proteins on platelets.

► Antiplatelet Drugs

Vomiting, Act of

The act of vomiting is a complex process accompanied by several events apart from activation of the motor nerves and various voluntary muscles involved in the increase of intragastric pressure and evacuation of the stomach contents. Vomiting is preceded by a deep inspiration, closure of the glottis and raising of the soft palate to prevent vomitus entering the trachea and nasopharynx, respectively. There is also increased heart rate, pallor, salivation, sweating and lacrimation.

► Emesis
► Serotonin

vWf

► von Willebrand Factor

VZV

Varizella Zoster Virus.

► Antiviral Drugs

Wall Peptidoglycan Inhibitors

- ▶ [β-Lactam Antibiotics](#)

Water Channel

Synonyms

Water Channels

- ▶ [Table appendix: Membrane Transport Proteins](#)
- ▶ [Aquaporins](#)

Watson-Crick Base Pairing

The rules of base pairing (or nucleotide pairing) are:

A with T: the purine adenine (A) always pairs with the pyrimidine thymine (T)

C with G: the pyrimidine cytosine (C) always pairs with the purine guanine (G).

This is consistent with there not being enough space (20 °) for two purines to fit within the helix and too much space for two pyrimidines to get close enough to each other to form hydrogen bonds between them. These relationships are often called the rules of Watson-Crick base pairing.

- ▶ [Antisense Oligonucleotides](#)

Weight-loss Therapies

- ▶ [Anti-obesity Drugs](#)

Wernicke's Syndrome/ Encephalopathy

Wernicke's syndrome is a serious consequence of alcoholism and thiamine (vitamin B₁) deficiency. Certain characteristic signs of this disease, notably ophthalmoplegia, nystagmus, and ataxia, respond rapidly to the administration of thiamine but to no other vitamin. Wernicke's syndrome may be accompanied by an acute global confusional state that may also respond to thiamine. Left untreated, Wernicke's syndrome frequently leads to a chronic disorder in which learning and memory are strongly impaired. This so-called Korsakoff's psychosis is characterized by confabulation, and is less likely to be reversible once established.

- ▶ [Drug Addiction/Dependence](#)

Wheal

The classical skin response to local release of histamine that results from contact with an allergen, irritant or following an insect bite. A central wheal develops as a direct result of local inflammation and the the oedema the follows the increased capillary permeability caused by histamine acting on H1-receptors on vascular endothelial cells.

- ▶ [Histaminergic System](#)

Wheezing

Continuous, coarse, whistling sound produced in the respiratory airways during breathing when air flow becomes accelerated through narrowed or obstructed airways.

- ▶ [Bronchial Asthma](#)

Wingless

a class of secreted morphogens that are central regulators of embryonic patterning processes.

- ▶ Low-Density Lipoprotein Receptor Gene Family
- ▶ Wnt Signaling

Withdrawal

The abrupt cessation of a repeatedly or continuously administered opioid agonist, or the administration of an antagonist typically results in the withdrawal syndrome. Signs and symptoms include sweating, tachycardia, hypertension, diarrhea, hyperventilation, and hyperreflexia.

- ▶ Drug Addiction/Dependence
- ▶ Analgesics

Wnt Signaling

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Synonyms

Int-1; Wingless, wg

Definition

Wnts are secreted proteins with important signaling roles in the development of all multicellular animals. Wnt signaling can be divided into two major types: ▶ “canonical” β -catenin-dependent signaling, and ▶ “non-canonical” non- β -catenin signaling, which itself is further divisible into multiple subtypes. Wnt signaling helps regulate cell fate specification, cell morphology, cell proliferation, cell migration, cell polarity, and tissue patterning in both ▶ vertebrates and ▶ invertebrates; misregulation of Wnt signaling is linked to some developmental disorders and too many forms of cancer.

Basic Mechanisms

Wnt takes its name from the *wingless* (*wg*) gene of the fruitfly *Drosophila melanogaster* (one hypomorphic mutation of the gene produces an adult fly without wings) and the *int-1* proto-oncogene, a target of MMTV, the mouse mammary tumor virus (which causes abnormal gene activation when it integrates into the host genome). The discovery that *Drosophila wg* and mouse *int-1* are orthologs and are part of a large conserved family of genes led to the contracted name “*Wnt*.”

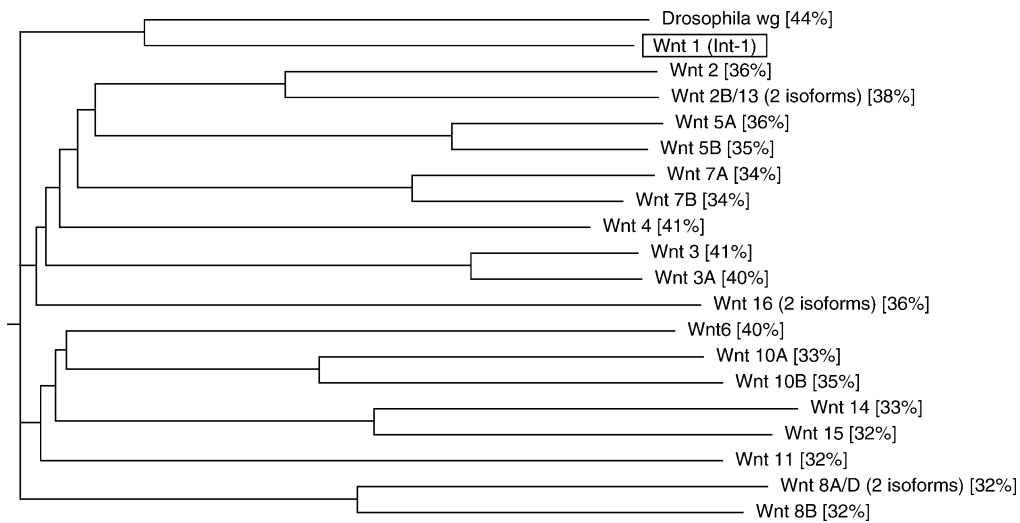
Wnt Structure and Biochemistry

There are 7 related *Wnt* genes in *Drosophila* and 19 in humans that generally have close orthologs in mice (Fig. 1). Orthologous *Wnt* gene-products are often very highly conserved between species.

The length of vertebrate Wnt protein precursors varies from about 100 amino acids to over 400 amino acids. All Wnt proteins contain a cleavable amino-terminal signal peptide that targets them for secretion, a conserved cysteine-rich composition, and multiple N-linked glycosylation sites. Wnt proteins synthesized in vivo are glycosylated and palmitoylated, rendering them very hydrophobic. Once outside the cell, mature Wnt glycoproteins associate with extracellular matrix and bind to ▶ heparin sulfate proteoglycans which help to stabilize and regulate their distribution. The secreted Wnt activates a ▶ Wnt receptor complex on target cells, leading to downstream signaling effects. In *Drosophila* non-*wg* producing cells endocytose *wg* that has been secreted by neighboring cells. Genetic studies have demonstrated that this endocytic activity is important both for facilitating *wg* signaling and for regulating its distribution.

Autocrine signaling by Wnt proteins may be important, both as a feedback mechanism during normal biological processes and in pathogenesis. Tumors induced by MMTV are clonal, suggesting that once the virus activates the *Wnt1* gene in a single mouse mammary gland cell, Wnt secretion stimulates uncontrolled proliferation in that cell and possibly other oncogenic changes as well. Similarly, some tissue culture cell lines transfected either with a *Wnt* gene or with DNA from MMTV-induced tumors become transformed and clonally tumorigenic. In *Drosophila*, there is evidence that autocrine signaling through *wg* activates a signaling cascade that is genetically partly distinct from paracrine *wg* signaling.

It has been proposed that the vertebrate *Wnt* genes can be classified based on whether they activate β -catenin-dependent or non- β -catenin signaling pathways (see below). However, the response to a Wnt of one proposed class can be switched to that of the other class by coexpressing a specific Wnt receptor.



Wnt Signaling. Figure 1 A sequence relatedness tree among the 19 human *Wnt* gene-products. *Drosophila wg* is included for comparison. The percent of amino acid residues in each protein that are identical to Wnt1 (boxed) is indicated. Wnt1/int-1 was chosen as the index sequence for purely historical reasons (it is not evolutionarily ancestral to the other Wnts). This tree is based on overall identity among predicted *Wnt* gene-products and is **not** meant to convey absolute evolutionary relationships. Horizontal distance from a node correlates with degree of sequence divergence; vertical distance is arbitrary. Sequences were obtained from the protein database of the National Center for Biotechnology Information and compared using the default matrix settings of MacVector 6.5.3 (Oxford Molecular Group). A non-Wnt sequence (FGF9) was used to root the tree (not shown).

Similarly, in developing *Drosophila* the response to wg is influenced by the relative abundance and ligand affinity of receptors expressed in the target tissue. A synthesis of the available data from all species suggests that the response to a specific Wnt signal in vivo is influenced both by the particular Wnt protein secreted and by the receptors and other downstream molecules present in the target tissue.

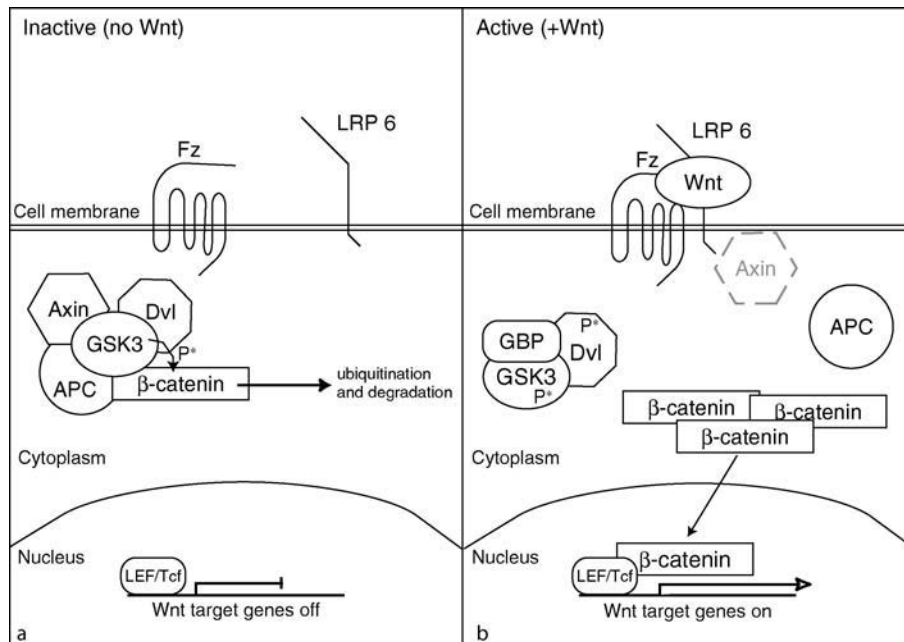
Wnt/ β -catenin-Dependent (“Canonical”) Signaling

The Wnt/ β -catenin-dependent pathway is typified in vertebrates by responses to a subclass of the vertebrate Wnt proteins and **▶Frizzled (Fz) receptors**, plus **▶LRP5 or 6**; in *Drosophila* it is typified by wg, *Drosophila* Frizzled 2 (DFz2), and the *Drosophila* LRP homolog (“arrow”). The central feature of this pathway is a multi-protein degradation complex that controls cytoplasmic concentrations of the multi-functional protein β -catenin. β -catenin plays a structural role at the inner cell membrane of adherens junctions, but also acts as a transcriptional coactivator in the nucleus. The concentration of soluble β -catenin in the cytoplasm and nucleus is determined by its rate of degradation through ubiquitination-dependent targeting to the proteosomal pathway. **▶Glycogen synthase kinase-3** (GSK3; particularly isoform GSK3 β) regulates this process by phosphorylating β -catenin, targeting it for degradation. Besides GSK3 itself, other components of the degradation complex include **▶Dishevelled** (Dvl), Axin, and

Adenomatosis Polyposis Coli (APC). Whereas Dvl is a Wnt pathway activator, GSK3, Axin and APC are all inhibitors. Axin and APC help to stabilize the activity of GSK3, keeping β -catenin levels low and Wnt target gene transcription off in the absence of a Wnt signal (Fig. 2a).

During Wnt/ β -catenin-dependent signaling several molecular events occur: (i) Dvl becomes phosphorylated (several different kinases, including Casein Kinase I (CK1), Casein Kinase 2 (CK2), and the Par1 kinase from *Drosophila* have been implicated in this). (ii) Axin dissociates from the degradation complex, translocates to the inner plasma membrane, and becomes less stable. (iii) Another protein called GSK3 Binding Protein (GBP or FRAT) binds both GSK3 β and Dvl. (iv) GSK3 β is phosphorylated, inhibiting its kinase activity. The net effect of these events is to destabilize the degradation complex, leading to phosphorylation/inactivation of GSK3 β . This prevents ongoing GSK3-mediated phosphorylation of β -catenin. Unphosphorylated β -catenin, which is not ubiquitinated nor targeted to the proteasome, then accumulates. In the nucleus, this free β -catenin acts with members of the LEF/TCF family of transcription factors to regulate expression of Wnt target genes (Fig. 2b).

Besides the well-established Wnt/ β -catenin-dependent pathway components mentioned above, several additional cytoplasmic inhibitors of Wnt/ β -catenin-dependent signaling have been identified that share



Wnt Signaling. Figure 2 Wnt/β-catenin-dependent signaling. (a) Inactive (no Wnt). (b) Active (+Wnt). Activation of a Fz/LRP6 receptor complex by a Wnt causes phosphorylation of Dvl and Gsk3, translocation of Axin to the membrane, and association of GSK3 with GBP. This downregulates GSK3-mediated phosphorylation of β-catenin, which normally leads to degradation of free β-catenin. Unphosphorylated free β-catenin accumulates in the cytoplasm and nucleus where it acts as a transcription coactivator with members of the LEF/TCF gene family.

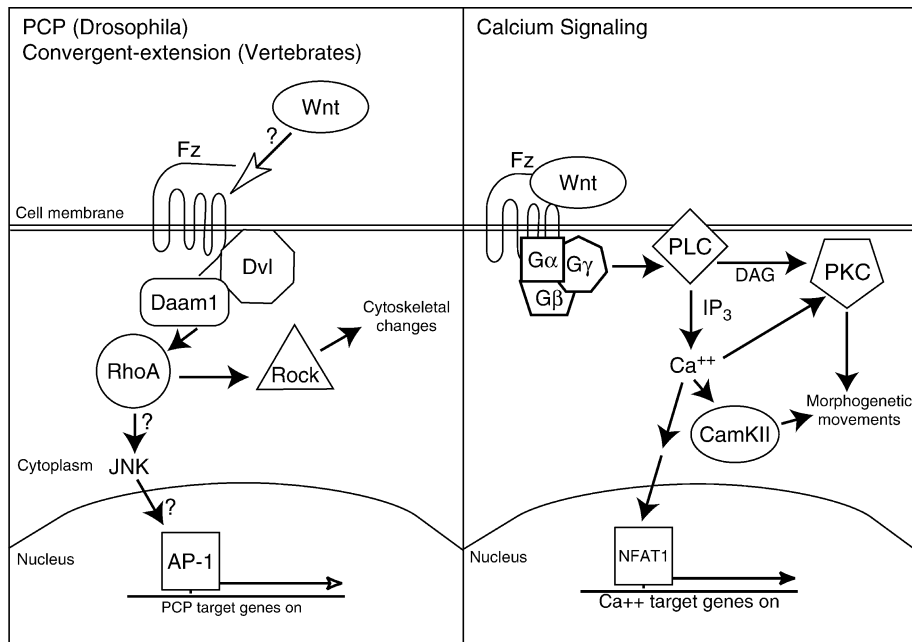
the ability to bind Dvl. These include the *Drosophila* gene *naked cuticle* (*nkd*) and the vertebrate proteins Idax and Dact (Dpr/Frodo). In the case of Dact there is conflicting evidence that it may act positively in Wnt/β-catenin-dependent signal transduction. In general, proteins that interact directly with Dvl, such as Nkd and Dact, also play roles in Wnt/non-β-catenin signaling (see below). There are also several secreted extracellular inhibitors of Wnt/β-catenin-dependent signaling that are biologically important for development and disease. These include cerberus (*cer*) and secreted Frizzled Related Proteins (sFRPs), which bind Wnt and therefore act as general Wnt inhibitors, Dickkopf (*dkk*), which binds to LRP6 and is therefore specific for Wnt/β-catenin-dependent signaling, and Wnt Inhibitory Factor-1 (WIF-1), which resembles the LRP extracellular domain.

Wnt/non-β-catenin ("Non-canonical") Signaling

Wnt/non-β-catenin signaling is not as well characterized biochemically as the Wnt/β-catenin-dependent pathway; this may reflect that it is molecularly more diverse, at least in vertebrates. In *Drosophila* the best characterized Wnt/non-β-catenin pathway is **planar cell polarity (PCP) signaling**. Ironically, although it certainly depends on a Fz receptor, it remains

controversial whether PCP signaling actually involves a Wnt ligand. Despite the fact that *wg* has been shown to bind Fz, there is no conclusive evidence that the endogenous ligand for Fz during PCP signaling is *wg*. The PCP pathway downstream of Fz has been defined on the basis of a combination of genetic and molecular data. As with Wnt/β-catenin-dependent signaling, the *Drosophila* Dvl homolog (*dsh*) is involved, although the requirement for conserved subdomains of the *dsh* protein and its subcellular localization differ in the two pathways (see glossary and Fig. 3a). Also, as with Wnt/β-catenin-dependent signaling, several *dsh*-interacting proteins have been identified that participate in PCP signaling, including the putative calcium-sensitive protein *nkd* and the Formin homology protein *Daam1*, which also binds the small GTPase RhoA. RhoA activates the *Drosophila* Rho-associated kinase (ROCK), which causes cytoskeletal changes by phosphorylating the nonmuscle myosin regulatory light chain and possibly other proteins (Fig. 3a).

PCP signaling has also been associated with activation of Jun-N-terminal Kinase (JNK), which can alter gene expression through the AP-1 transcription factor. However, the physiologic significance of this for endogenous PCP signaling remains unclear because JNK activation is a fairly nonspecific phenomenon in signaling biology.



Wnt Signaling. Figure 3 Wnt/non- β -catenin signaling. (a) PCP/Convergent-Extension signaling. Signaling through Fz causes Dvl to translocate to the inner plasma membrane. Dvl, with the RhoA-binding protein Daam1, activates the small GTPase RhoA. RhoA has multiple signaling outputs, including Rho-Associated Kinase (ROCK), which can directly regulate cytoskeletal dynamics. The JNK pathway may also be a downstream PCP effector. Multiple molecules not shown here are also implicated (see text). (b) Wnt/Calcium Signaling. Vertebrate Wnt5a and Wnt11 cause a transient increase in intracellular calcium, probably through inositol triphosphate (IP₃) and diacylglycerol (DAG) released by Phospholipase C (PLC). Calcium and DAG activate Protein Kinase C (PKC), Calcium/Calmodulin-regulated Kinase II (CamKII), and the NFAT1 transcription factor. The role of heterotrimeric G-proteins associated with the Fz receptor complex in Wnt pathways is still unclear, but most seven-pass transmembrane receptors are coupled to downstream signaling pathways in this way. The pathways presented in 3a and 3b may be alternate facets of a single conserved Wnt/non- β -catenin pathway, but there may also be a variety of Wnt/non- β -catenin signaling pathways, especially in vertebrates (see text).

Aside from the PCP pathway proteins described above, several other genetically identified components play roles that are not yet understood at a biochemical level. Although arrow/LRP6 is not required for PCP signaling, at least three other transmembrane proteins have been implicated that may act independently of, or even antagonistically to, Fz receptors: flamingo (fmi), strabismus (stbm or vang), and fuzzy (fy). Also implicated is the membrane-associated daschous (ds) protein, a member of the cadherin superfamily. Cytoplasmic proteins that play a role include the gene-products of *prickle* (*pk*), *inturned* (*in*), and *multiple wing-hair* (*mwh*). How these proteins connect to other components of the PCP pathway is not yet known, but the Wnt/non- β -catenin signaling function of at least some of them is conserved in vertebrates. Homologs of these proteins have been implicated in “convergent-extension” movements during vertebrate development. This suggests that the same Wnt/non- β -catenin signaling pathway controls

aspects of cell polarity in *Drosophila* and cell shape and movement in vertebrate embryos.

Another non- β -catenin pathway antagonizes β -catenin-dependent Wnt signaling and is typified by responses to Wnt5a and Wnt11 in vertebrate cells. The main characteristic of this pathway is IP₃-mediated rapid increase of intracellular calcium followed by activation of Protein Kinase C (PKC), Calcium/Calmodulin-regulated Kinase II (CamKII), and the NFAT1 transcription factor (Fig. 3b). There is considerable evidence that initial signaling downstream of the Fz receptor complex in this [Wnt/Calcium signaling pathway](#) occurs through [heterotrimeric G-proteins](#). Studies in mammalian cultured cells and in *Xenopus* embryos have demonstrated that Wnt5a activates the JNK cascade, providing a possible link between the vertebrate Wnt/Calcium signaling pathway and the *Drosophila* PCP pathway. Furthermore, activators of the Wnt/calcium pathway (e.g., Wnt5a) and of PCP signaling (e.g., Stbm and Nkd) similarly

affect convergent-extension movements during vertebrate gastrulation. One attractive hypothesis is that the Wnt/Calcium and PCP signaling pathways are two aspects of the same conserved molecular cascade. This remains speculative in part because the Wnt/Calcium signaling pathway has been characterized primarily through cell biological approaches in vertebrates, whereas the PCP pathway has been characterized primarily through genetic approaches in *Drosophila*.

Finally, among the most unexpected recent Wnt signaling findings is that in response to wg stimulation at the *Drosophila* neuromuscular junction, the intracellular domain of the DFz2 receptor is cleaved and translocates to the nucleus. It is unclear what function this DFz2 fragment serves, or how widespread this potential mechanism of Wnt signal transduction might be.

LRP5 and 6 are not required for Wnt/non- β -catenin signaling; instead a protein called **Ryk** (derailed in *Drosophila*) is the best characterized coreceptor for the Fz proteins in non- β -catenin signaling. Ryk/derailed was first implicated in axon guidance, but could conceivably play a more general role in all forms of Wnt/non- β -catenin signaling controlling cell shape, movement, and polarity.

Wnts and Cancer

Alteration in the Wnt/ β -catenin-dependent signaling pathway is now a well-established etiologic agent in oncogenesis. The evidence is most striking for two downstream intracellular signaling components: the gene-product of the *APC* locus, and the β -catenin protein. APC is a negative regulator of Wnt/ β -catenin-dependent signaling that is mutated in the majority (>80%) of sporadically occurring colorectal adenomas and carcinomas. In addition, *APC* mutations cause the genetic disease Familial Adenomatous Polyposis, characterized by proliferation of initially benign colonic polyps that greatly predispose afflicted individuals to intestinal cancer. Similarly, mutations in β -catenin have been described in many different cancer types. Mutations in other Wnt pathway components have likewise been associated with cell transformation in vitro and with pathogenesis of an array of cancer types in vivo (Table 1). A general model is that inappropriate activation of Wnt/ β -catenin-dependent signaling alters gene expression, leading to abnormalities in cell fate and proliferation.

Interestingly, the role of Wnt/non- β -catenin signaling in controlling cell morphology and movement may contribute to another important aspect of neoplasticity:

Wnt Signaling. Table 1 Some of the major Wnt signaling components^a in human cancer

Wnts	sFRPs	Dact1	Axin	APC
Breast cancer (WNT1, WNT5a)	Breast cancer	Invasive breast cancer	Liver cancer	Colorectal cancer (spontaneous)
	Colorectal cancer	Liver cancer		
	Bladder cancer			Familial Adenomatous Polyposis (genetic pre-cancerous condition)
	Prostate cancer			
Leukemia (WNT16)				
Lung cancer (Wnt7a)				
Fzs	Dkk	β -catenin	Dvl1	WIF-1
Lung (Fz9)	Multiple myeloma	Colorectal cancer	Skin cancer	Leukemia
				Breast cancer
Liver (Fz7)	Colorectal cancer	Liver cancer		Gastrointestinal cancer
		Ovarian cancer		Lung cancer
		Pancreatic cancer		
		Prostatic cancer		
		Skin cancer (melanoma)		
		Stomach cancer		
		Uterine cancer		

^aSee text for full molecule names and roles in Wnt signal transduction.

metastasis. There is growing evidence that genes involved in Wnt/non- β -catenin signaling are specifically misregulated in tumors as they increase in stage and become more invasive and aggressive.

Wnts and Other Diseases

Given the prevalence and biological significance of Wnt signaling, it should not be surprising that it has also been linked to many other diseases. A rare childhood disorder of bone formation, Osteoporosis-Pseudoglioma Syndrome, is caused by a genetic disruption in Wnt/ β -catenin-dependent signaling. Disruptions in Wnt signaling underlie an assortment of other diseases as well (Table 2). There is emerging evidence that Wnt signaling contributes to pathogenic processes ranging from scar formation to heart failure to neuropsychiatric disorders, and it is also an important signaling pathway in stem cell biology. Research into Wnt signal transduction therefore can lead to therapies that may 1 day help alleviate a corresponding variety of medical problems.

Pharmacological Intervention

Direct pharmacological manipulation of Wnt signaling (i.e., by activating Fz and/or LRP receptors or by inhibiting the Wnt/Fz interaction) by small-molecule drugs is not yet possible; large peptide ligands such as Wnts are difficult to mimic with the small, relatively nonpolar molecules that make good drugs. However,

native protein inhibitors of Wnt signaling, such as sFRP and Dkk proteins, may eventually be used to inhibit Wnt signaling in vivo. Similarly, expression of other negative regulators of Wnt signaling such as APC, Axin, and related proteins may prove useful in cancer treatment. Antisense oligonucleotides and RNA interference strategies targeting Wnt signaling components are under investigation both in pharmaceutical companies and in academic laboratories.

Other molecules that participate in Wnt-activated signaling pathways are pharmacologically accessible by more traditional treatments. The antitumor agent Gleevec/STI-571 has been shown to downregulate Wnt/ β -catenin-dependent signaling, as have retinoids, implying a link between Wnt and retinoic acid signaling. Fumagillin, an antitumor drug that acts as an angiogenesis inhibitor, has been shown to block Wnt/non- β -catenin signaling. Nonsteroidal antiinflammatory drugs (NSAIDs) that inhibit the cyclooxygenases COX 1 and 2 may also prove useful; these proteins are implicated in many human cancers and contribute to aberrant Wnt signaling in ways that await full elucidation.

In noncancer-related pharmacology, GSK3 is inhibited by lithium at therapeutic concentrations, implying that the long-established effectiveness of lithium in the treatment of psychiatric mood disorders (and more recently as a neuroprotective agent) may be linked to GSK3 inhibition. Antipsychotics such as haloperidol

Wnt Signaling. Table 2 Some of the major Wnt signaling components^a implicated in human disease

Wnt Signaling (general)	Wnt4	Sfrp	Fz	LRP5	Dvl	GSK3	β -catenin
Polycystic kidney disease (Polycystin-1 activates canonical Wnt signaling pathway)	Injury-induced renal fibrosis	Heart failure	Ulcerative colitis	Osteoporosis-Pseudoglioma Syndrome (genetic syndrome of defective bone formation)	Ulcerative colitis	Familial Alzheimer's disease (through interaction with Presenilin-1)	Familial Alzheimer's disease (through interaction with Presenilin-1)
	Wound healing				Schizophrenia (mouse knock-out model)		
				Other bone-growth syndromes		Schizophrenia (reduced levels in prefrontal cortex)	
						Bipolar Disorder (enzymatic activity reduced by therapeutic agents)	

^aSee text for full molecule names and roles in Wnt signal transduction.

have also been shown to change Wnt signaling in the brain, suggesting a link between neurotransmitter receptors (such as their target dopamine receptors) and downstream Wnt signaling components. Since many neurotransmitter receptors are members of the seven-pass transmembrane receptor superfamily that includes the Fz proteins, this raises the interesting possibility that a common mechanism might mediate interactions between cell surface receptors and Fz-interacting proteins such as Dvl in neurons.

The indisputable role of Wnt signaling in cancer pathogenesis ensures that molecules capable of altering Wnt signal transduction will continue to be a subject of intense pharmaceutical interest. Technological advances in the pharmacologic manipulation of these signaling pathways are sure to lead to important discoveries about Wnt signaling in a wide array of basic biological and clinically relevant processes.

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Wnts and Vertebrate Development

The first clue to a role for Wnt/ β -catenin-dependent signaling in vertebrate development came from over-expression studies in the classical embryonic system, the frog *Xenopus laevis*. When Wnt1 is ectopically expressed in cells that would normally contribute to the ventral embryo (i.e. the future belly), they instead adopt

a dorsal and anterior fate (i.e. they become future head). This induction of a “secondary dorsal axis” produces a striking phenotype: two-headed tadpoles. The ability of ectopic Wnt1 to induce a secondary dorsal axis derives from the participation of Wnt/ β -catenin-dependent signaling in dorsal cell fate specification early in vertebrate embryogenesis. However it remains unclear whether an endogenous intercellular Wnt signal, or only the downstream intracellular signaling cascade, is normally responsible for early dorsal fate determination. Nonetheless, experimental analyses across vertebrate model organisms have established the importance of Wnt signaling in a broad spectrum of developmental events.

► Wnt Signaling

Wortmannin

Wortmannin, a metabolite of the fungus *Penicillium funiculosum*, is a specific inhibitor of phosphoinositide 3-kinases (PI3Ks) with an inhibitory concentration (IC₅₀) determined in vitro of around 5 nM, making it a more potent inhibitor than LY294002, another commonly used PI3K inhibitor. It displays a similar potency in vitro for the class I, II and III PI3K members although it can also inhibit other kinases.

► Phospholipids

WW Domain

Protein-protein interaction domain which, like SH3 domains, bind to polyproline sequences.

► Adaptor Proteins

Xanthine Oxidase

Xanthine oxidase (XOD) is the key enzyme in purine catabolism. XOD catalyses the conversion of hypoxanthine to xanthine and of xanthine to uric acid, respectively. The uricostatic drug allopurinol and its major metabolite alloxanthine (oxypurinol) inhibit xanthine oxidase.

► Anti-gout Drugs

Xanthines

Derivatives of 2,4-dioxopurine, which may act as adenosine receptor antagonists, depending on the chemical structure.

► Adenosine Receptors
► Anti-gout Drugs

Xenobiotic

Xenobiotics are chemicals not naturally belonging to or originating from a particular organism or an ecosystem (from Greek *xenos*: foreign). Environmental pollutants like agricultural pesticides, food constituents, especially those produced by charbroiling, many drugs, cosmetics are typical xenobiotics. The drug-metabolizing system of vertebrates provides a chemical protection aimed at detoxifying xenobiotics.

► P450 Mono-oxygenase System
► MDR-ABC Transporters

X-linked Nephrogenic Diabetes Insipidus

X-linked nephrogenic diabetes insipidus (NDI) is caused by mutations in the gene for the vasopressin V2 receptor leading to an insensitivity of the kidney for the antidiuretic hormone arginine vasopressin (AVP). The main symptom of the disease is diuresis, i.e., the production of a large amount of diluted urine. Due to the massive loss of water, the patients suffer from thirst and are in danger of dehydration. The disease usually becomes evident shortly after birth.

► Protein Trafficking and Quality Control

X-ray Contrast Agents

► X-ray Contrast Agents and Molecular Imaging

X-ray Contrast Agents and Molecular Imaging

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Synonyms

Radiocontrast agents; X-ray contrast agents; Contrast media; Contrast materials

Definition

Molecular imaging modalities are being developed with the desire for earlier detection of abnormalities before they develop into diseases, earlier detection of responses to therapy and more precise and specific information on the quality and site of disease processes. According to a consensus of a group of experts molecular imaging refers to techniques that serve to “directly or indirectly monitor and record the spatio-temporal distribution of molecular or cellular processes” [1]. Although MR spectroscopy detects and quantifies endogenous biomolecules and thereby allows evaluation of molecular and cellular processes, the basic idea is to achieve the goal by administration of visible pharmaceuticals that distribute in the living organism and either accumulate in specific areas and/or generate or change a signal which can be detected by imaging equipment.

The purpose of this article is to discuss whether and how X-ray contrast media may contribute to molecular imaging.

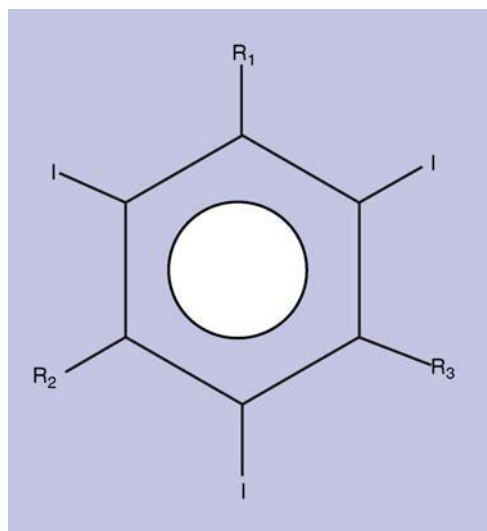
Mechanism of Action

Basic Properties of X-ray Contrast Agents

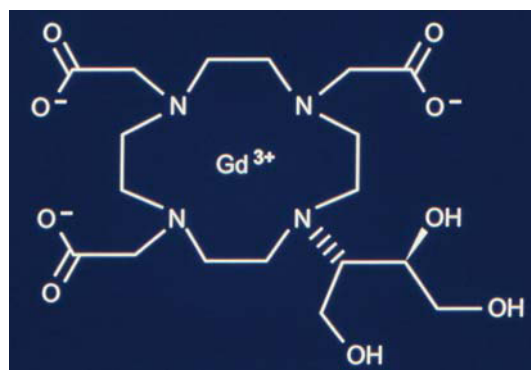
X-ray contrast agents absorb X-rays either more or less strongly than the usual body constituents, primarily water. Gas has a low density and therefore does not absorb X-rays to an extent that would be relevant to medical imaging. Nevertheless, gas is still used to outline body cavities but in most cases is replaced by dense compounds containing heavy elements. The most commonly used X-ray contrast agents are water-soluble triiodinated derivatives of benzoic acid (Fig. 1). This class of compounds was developed during a selection process which began with the discovery of X-rays. Over the last 60 years all new X-ray contrast molecules belonged to this class of compounds. Only after the introduction of metal chelates (Fig. 2 and 3) as contrast agents for MRI, elements other than iodine were reconsidered for use as X-ray contrast media. Up to now, however, none of the chelates has been approved for X-ray imaging.

Whereas imaging agents that can serve to detect molecular and cellular processes must in some way directly or indirectly participate in these processes or interact with components participating in the processes, the development of X-ray contrast agents led in the opposite direction: Molecules displaying the least possible interaction with body constituents and processes were preferred because of advantages in tolerability [2].

X-ray contrast media are usually highly concentrated aqueous solutions. A typical product contains 300–370 mg iodine/ml, which corresponds to about 600–800 mg of the iodinated organic molecule per ml. The high concentration of the contrast substance leaves limited room for water (about 0.7 ml per ml of contrast agent) and restricts the design of the molecules because

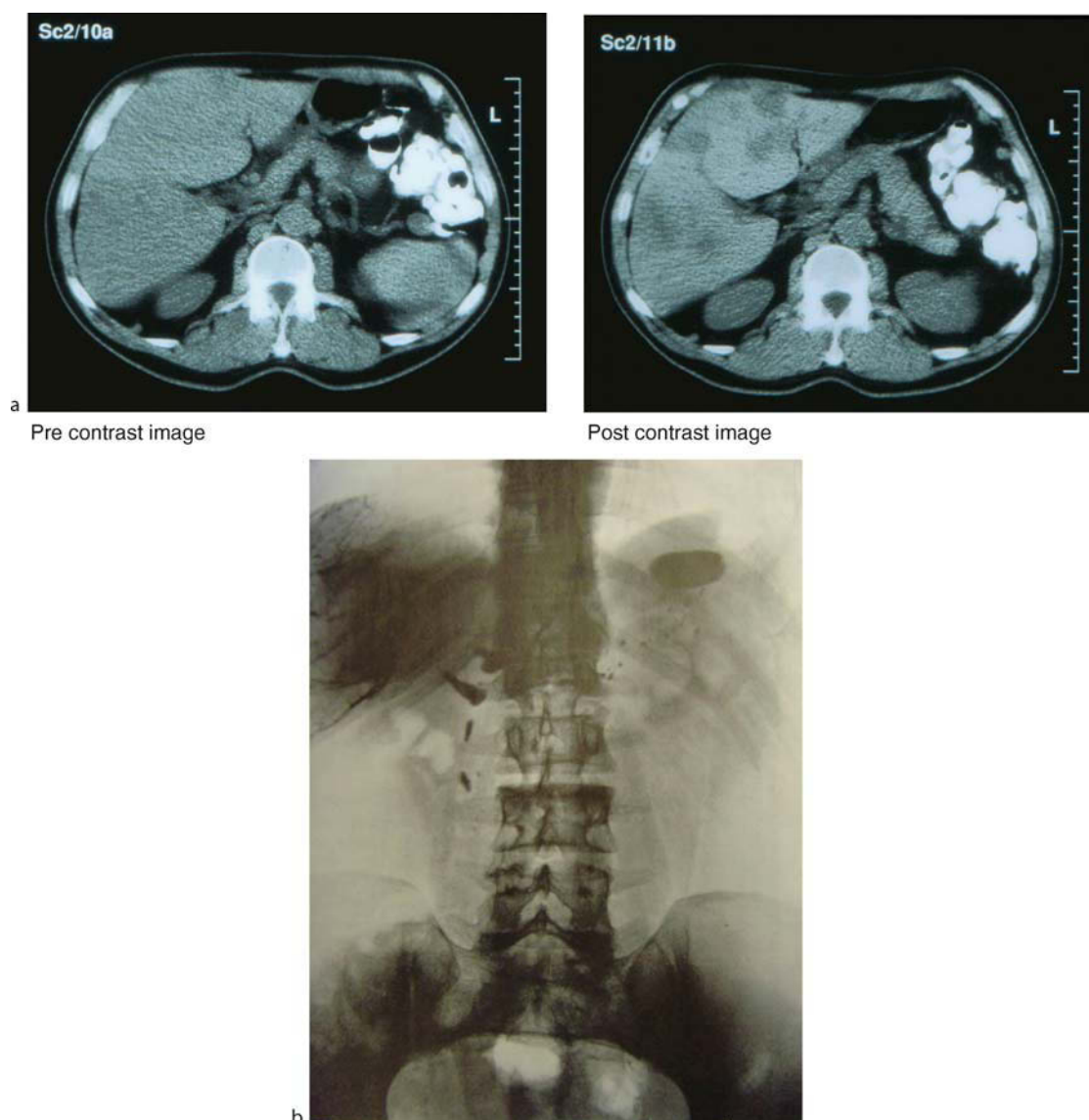


X-ray Contrast Agents and Molecular Imaging.
Figure 1 Basic chemical structure of water-soluble iodinated X-ray contrast agents.



X-ray Contrast Agents and Molecular Imaging.
Figure 2 Gadobutrol, a metal chelate used in magnetic resonance imaging. Metal content: 26% of weight.

limitations regarding osmolality and viscosity of the final preparation must be observed in addition to solubility, chemical stability, and cost. One important restriction is that an extremely high content of iodine or other heavy element is required in the molecules. The iodine content of molecules in currently available X-ray contrast media is in the range of 44–49%. It is a drawback of metal chelates that their metal content reaches only half this value. The larger the proportion of the organic constituents contributing very little to X-ray absorption, the less room remains for water, which further increases viscosity and osmolality. Due to their very high iodine content, X-ray contrast agents have a specific weight of up to 1.4 g/ml which is much higher than that of any biological tissue.



X-ray Contrast Agents and Molecular Imaging. Figure 3 Examples of X-ray contrast agents that enhance contrast due to intracellular uptake. (a) Liver enhancement following intravenous administration of a lanthanide chelate. (b) Visualization of e.g., lymph nodes following intravenous injection of colloidal thorium oxide. The agent is severely toxic but the example indicates that specific uptake of radiopaque compounds can be sufficient to opacify small structures in conventional radiographs (Börner et al (1960) Zur Problematik der Thorotrastschäden. Klinische und radiologische Untersuchungen zum Verhalten von Thorium und seiner Zerfallsprodukte im Organismus. Fortschr Röntgenstr 93:287–297).

Sensitivity

X-ray imaging can be used throughout the body. X-rays penetrate all body constituents with modest scattering, which allows precise localization of objects deep in the tissue.

X-ray absorption is a well understood process. It provides precise quantification of e.g., contrast agent concentration in the tissue or body cavities and excellent spatial and temporal resolution. Diagnostic applications do not require advancing the X-ray source

or detector into body cavities by endoscopic methods as with optical imaging or when special coils are used to improve image quality in MRI.

Most disease-specific molecular and cellular processes involve low concentrations and have low capacities. Therefore, molecular imaging requires highly sensitive imaging modalities to detect and quantify the imaging probes as it is done with short-lived radioisotopes. Optical imaging and ultrasound are next in sensitivity. MRI would require too high contrast agent concentrations

to allow detection of biomolecules, e.g., by labeled antibodies, or to trace intracellular metabolism.

The most relevant drawback of X-rays is their low contrast sensitivity. Even in CT a minimum concentration of 0.5 mg iodine/ml is required to achieve a detectable change of about 10–15 HU and ten times this concentration would be desirable. 0.5 mg iodine/ml corresponds to a 1–4 mM concentration of a molecule labeled with 1–3 iodine atoms.

Examples of X-ray contrast agents which reach sufficient concentrations by active transport mechanisms are compiled in Table 1. The smallest dose is about 3 g iodine in case of ►cholegraphic agents and about 3 g gadolinium when Gd EOB DTPA is used for enhancement of liver parenchyma. Targeting smaller volumes of tissue than liver parenchyma may require less contrast material. Small lesions will, however, receive only a small proportion of cardiac output. If perfusion is in the same range as in best perfused normal tissue, i.e., 1 ml/g/min, it will take almost 10 h until the blood volume of 5 L passes a 10-ml lesion. Meanwhile competing processes will have diminished blood concentration. If extraction is 100% during one passage of the blood and contrast enhancement of 50 HU in the 10-ml tissue is desired this would mean a dose of 100 mg iodine or 500 mg contrast agent under optimistic assumptions, as described in following list.

Estimation of minimum dose necessary to achieve 50 HU contrast enhancement by a specifically binding or transported iodinated contrast agent

- 50 HU corresponds to 2 mg iodine/ml tissue.
- 10 g of tissue requires 20 mg iodine.
- 20 mg iodine is contained in 5 L of blood if a dose of 100 mg iodine is injected and the mean blood level for the next 10 h is 20% of the theoretical initial concentration (80% loss due to biodegradation, diffusion into tissues, excretion; most likely losses will be higher).
- 100% extraction of the contrast agent during first pass, 10 h of perfusion.
- If the specific contrast agent molecule contains 20% (weight) iodine the total dose required is 500 mg.

Improvements in contrast sensitivity require higher radiation doses or the application of lower energy X-rays, both are not acceptable for most patients and indications. Monoenergetic X-rays or X-rays with narrow energy distribution make better use of the sudden rise in absorption occurring at the k-edge of iodine and other heavy elements but none of these measures would result in dramatic improvements regarding sensitivity. Consequently, molecular imaging

using X-ray imaging can be used only for the detection of high capacity molecular or cellular processes, the detection of molecules which occur in high concentrations, or processes which result in physicochemical states that lead to accumulation of contrast agent molecules independently of an active transport or specific binding.

Specificity of Extracellular X-ray Contrast Agents

Although frequently called “nonspecific” the currently dominating extracellular X-ray and MR contrast agents display very useful specificities for a large variety of diseases. The contrast media are distributed throughout the body by the blood flow and diffusion according to the permeability of capillaries and the size of the interstitial space [3]. Perfusion, capillary permeability, and the proportion of extracellular space differ in ischemic states, inflammation, and tumors [4]. The faster the imaging becomes and the larger the volumes simultaneously scanned, the better the early distribution phase of rapidly injected contrast media is displayed. This early distribution allows best to differentiate perfusion- and diffusion-depending differences in contrast. As scanners were much slower in the past, the potential benefits of the extracellular contrast media were not fully recognized.

Limitations are obvious if the diseased tissue does not differ from normal tissue or successfully treated tissue in respect of the above-mentioned criteria. Under these conditions, even a contrast agent with high absorption of X-rays is of no help. Another drawback is the short-lasting contrast which requires repeated injections if the diagnosis is missed during the first scan or if persistent visualization of a lesion is required during an interventional procedure.

Monitoring of Molecular and Cellular Processes by X-ray Contrast Agents

Molecular and cellular processes were visualized by X-ray contrast agents long before the term “molecular imaging” became popular and even before the advent of early CT (Table 1). Because of the above-mentioned limitations in the sensitivity of contrast detection by X-ray radiography, the latter can only visualize high capacity transport mechanisms and the nonspecific uptake of particles by specialized cells.

None of the products or experimental preparations is currently in clinical use or under development. Thorium oxide was not excreted at all; furthermore it proved to be toxic because of long-lived α -radiation [4]. Other agents were not pursued because they displayed various types of toxicity or were less well tolerated than the extracellular contrast agents. Except thorium dioxide, none of them resulted in reliable and satisfactory contrast or provided important diagnostic information which could not have been obtained with a similar quality by more recently established imaging methods. In spite of an everlasting

X-ray Contrast Agents and Molecular Imaging. Table 1 Examples of X-ray contrast agents used to monitor molecular and cellular processes

Target	Contrast medium	Commercial product or experimental	Biological mechanism	Reference
Liver, spleen, lymph nodes	Thorium oxide	Thorotrast®	Uptake by macrophages, RES	Urich K. (1995) Successes and failures in the development of contrast media. Blackwell, Berlin
Liver, spleen	Liposomes, lipiodol emulsions, iodipamide particles	Experimental, phase I, II clinical trials	Uptake by macrophages, RES	(a) Weichert JP et al (2000) Combined hepatocyte-selective and blood-pool contrast agents for the CT detection of experimental liver tumors in rabbits. Radiology 216:865–871; (b) Leander P et al (1998) New liposomal liver-specific contrast agent for CT: first human phase I clinical trial assessing efficacy and safety. Acad Radiol 5(Suppl1):S6–S8; (c) Violante MR et al (1981) Biodistribution of a particulate hepatolienographic CT contrast agent: a study of iodipamide ethyl ester in the rat. Invest Radiol 16:40–45
Gall bladder	Various oral cystographic agents, e.g., iopanoic acid	Telepaque etc.	Anion transport	Urich K, Speck U (1991) Biliary excretion of contrast media. Progr Pharmacol Clin Pharmacol 8:307–322
Bile ducts	Various intravenous cholegraphic agents, e.g., iodipamide	Biligradin®	Anion transport	Lin SK et al (1977) Iodipamide kinetics: Capacity-limited biliary excretion with simultaneous pseudo-first-order renal excretion. J Pharm Sci 66:1670–1674
Urinary tract	Early ionic urographic agents including iodamide	Uromiro®	Tubular secretion	Difazio LT et al (1978) Pharmacokinetics of iodamide in normal subjects and in patients with renal impairment. J Clin Pharmacol 18:35–41
Pancreas	Oral cholecystographic agent iopanoate (?)	experimental	Unknown; excretion into pancreatic duct	Schmiedl U et al (1994) Imaging of exocrine pancreatic function investigation of the bioavailability of weak organic acids as potential pancreatic contrast agents for computed tomography. Invest Radiol 29:689–694
Liver	Gadolinium EOB DTPA	Primovist®; MRI product, not approved for CT; phase I, II clinical trials	Uptake into hepatocytes	Schmitz SA et al (1997) Detection of focal liver lesions: CT of the hepatobiliary system with gadoxetic acid disodium, or Gd-EOB-DTPA. Radiology 2002:399–405
Liver	Selected hepatobiliary agents	Experimental, phase I clinical trial	Uptake into hepatocytes	(a) Mützel W et al (1982) Water-soluble contrast agents for computed tomography of the liver: experimental studies in dog. In: Amiel M, Moreau JF (eds) Contrast media in radiology: appraisal and prospects. Springer, Berlin-Heidelberg-New-York, S, p 320–323; (b) Leander P et al (1998) New liposomal liver-specific contrast agent for CT: first human phase I clinical trial assessing efficacy and safety. Acad Radiol 5(Suppl1):S6–S8

interest in specific contrast agents one after the other of these specific contrast agents disappeared from the market.

Admittedly, none of the X-ray contrast agents that used to be labeled as specific were developed to solve important diagnostic problems that are of interest today such as early tumor detection or diagnosis of non-obstructive inflammatory arterial or degenerative cerebral disease.

Directions of Future Research

It is not very likely that X-ray contrast agents will be used for molecular imaging. The minimal concentration required is probably far too high to be reached by specific binding even if small molecules are used to direct iodine or other radiopaque elements to diseased tissue. Nevertheless, some metabolic or transport processes operate with high capacity. In these cases, the required concentration may be reached if the metabolized or transferred contrast agents accumulate in the cells, tissues, or cavities where the process takes place. Such accumulation may occur if the contrast agent is caught in the cells or cavity because of unidirectional transport or is biotransformed to charged or more hydrophilic, nondiffusible metabolites or there is precipitation because the concentration exceeds solubility.

Other mechanisms that indicate abnormalities but are less directly dependent on molecular or cellular processes may be used to achieve sufficient accumulation of X-ray contrast agents: There are several disease processes that result in changes of e.g., extracellular pH or accumulation of calcium or extracellular matrix materials [4,6]. It is conceivable that specifically designed contrast agent molecules accumulate by precipitation at acidic pH or formation of large aggregates or binding to calcium deposits. The process may be enhanced if the contrast agent molecules tend to bind to each other either already in aqueous solution or after they change their shape or ionicity following any kind of reaction or binding at the target location.

Although a variety of concepts seem to offer opportunities for the development of X-ray contrast media with specificities different from those of the currently used inert small molecules with extracellular distribution one has to consider the following obstacles:

- In the past 100 years many approaches tested intentionally or unintentionally have failed. Nevertheless, the underlying concepts may be tested again today with promise, applying current knowledge in biology and chemistry.

- The mechanisms that are most likely to result in sufficient specificity and contrast medium concentration involve complicated multiple step reactions.
- Toxicity of accumulating X-ray contrast agents requires specific attention.
- Cost caused by expensive development and production must be balanced against the medical benefit.

Increasing spatial and to some extent also contrast resolution of X-ray equipment and advances in the understanding of disease processes are in favor of improved X-ray contrast agents which provide important additional information.

Conclusions

The combination of molecular imaging and X-ray contrast agents sounds like an unresolvable contradiction. Extracellular, well-tolerated and cheap **▶nonionic contrast media** that allow us to assess perfusion, permeability, and the proportion of interstitial space do indeed set a standard that is difficult to reach or even surpass. Yet, X-ray contrast media meeting a broad definition of molecular imaging were used in the past. They failed because the processes displayed were of too little medical significance and/or tolerance was not satisfactory. An X-ray contrast agent indicating a universal process such as pH-lowering or extracellular matrix accumulation may find a place within the broad range of diagnostic imaging methods.

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Y-27632

A compound that lowers elevated blood pressure by inhibition of Rho-kinase.

► Smooth Muscle Tone Regulation

YAC

An yeast artificial chromosome (YAC) is a vector that allows the propagation of large exogenous DNA fragments, up to several megabases, in yeast.

► Transgenic Animal Models

Zellweger Syndrome

Fatal hereditary disorder that typically presents in the neonatal period. Clinical features include an array of hepatic, renal and neurological dysfunctions. Patients with Zellweger syndrome rarely survive the first year of life. The disease is caused by mutations in the Pex proteins leading to an defective import of peroxisomal matrix proteins and consequently to a loss of most peroxisomal metabolic pathways.

►Protein Trafficking and Quality Control

Zero-Order Kinetic

Zero-order kinetics describe the time course of disappearance of drugs from the plasma, which do not follow an exponential pattern, but are initially linear (i.e. the drug is removed at a constant rate that is independent of its concentration in the plasma). This rare time course of elimination is most often caused by saturation of the elimination processes (e.g. a metabolizing enzyme), which occurs even at low drug concentrations. Ethanol or phenytoin are examples of drugs, which are eliminated in a time-dependent manner which follows a zero-order kinetic.

►Pharmacokinetics

ZES

►Zollinger-Ellison Syndrome

Zinc Finger

Zinc fingers are structural motifs which were first recognized in DNA-binding domains of various proteins but which are now known to occur also in proteins that do not bind to DNA. In the classical C_2H_2 zinc finger domain, three secondary structures, an α -helix and two β -strands with an antiparallel orientation, form a finger-like bundle, which is held together by a zinc ion. Zinc ions are bound by 2 cysteine and 2 histidine residues, which are localized in the β -strands and the α -helix. The C_2H_2 zinc finger domain, which can insert its α -helix into the major groove of DNA, is one of the most common DNA-binding motifs in eukaryotic transcription factors. A second type of zinc finger structure, designated the C_4 zinc finger, is found in nuclear receptors. While C_2H_2 zinc finger proteins generally contains 3 or more repeating zinc finger motifs and bind as monomers to DNA, nuclear receptors contain only 2 C_4 zinc finger units and bind to DNA as homodimers or heterodimers. The C_4 zinc finger motif is structurally quite as distinct from the C_2H_2 zinc finger domain.

Zollinger-Ellison Syndrome

Zollinger-Ellison syndrome (ZES) is characterized by the development of a tumor (gastrinoma) or tumors that secrete excessive levels of gastrin, a hormone that stimulates production of acid by the stomach. In most cases, the tumor or tumors arise within the pancreas and/or the upper region of the small intestine (duodenum).

►Proton Pump Inhibitors

►Proton Pump Inhibitors and Acid Pump Antagonists

Zona Glomerulosa

The adrenal cortex is functionally divided into three zones, the zona glomerulosa, fasciculata, and reticularis. Only the outermost zone, the zona glomerulosa, synthesizes aldosterone. The other zones are responsible for the generation of glucocorticoids and androgens.

- ▶ Epithelial Na⁺ Channel (ENaC)
- ▶ Gluco-/Mineralcorticoid Receptors

Zymogen

Zymogen is a precursor protein that is converted to an active protease when one or more of its peptide bonds are cleaved. Zymogens involved in coagulation include factors II (prothrombin), VII, IX, X, and XI.

- ▶ Anticoagulants
- ▶ Coagulation/Thrombosis

List of Entries

Essays are shown in blue

2DE
14-3-3σ
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 Array
 Arrestins
 Arrhythmias
 Arrhythmogenic Right Ventricular Dysplasia/
 Cardiomyopathy
 Arteriogenesis
 Arteriosclerosis
 Arylhydrocarbon Receptor
 L-Ascorbic Acid
 ASF Family of Transporters
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 ASON
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 Basal Activity
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 BCRP
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 Benign Familial Neonatal Convulsions
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Bone Metabolism
Bone Morphogenetic Proteins
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Bradykinin
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 Erectile Dysfunction
 ERGIC
 Ergot Alkaloids
 ERT
 Erythropoietin
 ESI-MS
 EST
 Estrogen Receptor
 Estrogen Replacement Therapy (ERT)

Estrogens
[Ethanol](#)
Ethyl Alcohol
Euglycaemia
Eukaryotic Expression Cassette
Excitability
Excitation–contraction Coupling
Excitatory Amino Acids
Excitotoxicity
Exenatide
[Exocytosis](#)
Exogenous Pyrogens
Exon
Exopeptidase
Exportins
Extended Amygdala
Extracellular Calcium-sensing Receptor (CaSR)
Extracellular Matrix (ECM)
Extrapyramidal Side Effects
Extrasynaptic Receptors
F-actin
Fab Fragments
Factor IIa
FAD
Familial Alzheimer's Disease
Familial Hypocalciuric Hypercalcemia (FHH)
Fanconi-Bickel Syndrome
Farnesyl Transferase Inhibitors (FTIs)
FAS Ligand (FasL)
FAT10
[Fatty Acid Transporters](#)
Favism
Fc Receptor
[Fever](#)
Fibrates
A-fibres
Fibrin
Fibrinogen
[Fibrinolytics](#)
Fibroblast Growth Factors
Filamin
First-order Kinetics
First-pass (Presystemic) Metabolism
FK506
FKBP12
Flare
Flavin Adenine Dinucleotide (FAD)
Flavin Mononucleotide (FMN)
Flp/FRT
Fluorescence in situ Hybridization (FISH)
Fluoride
Fluoroquinolones
FMN
Foam Cells
Folate
[Folic Acid](#)
Follicle-stimulating Hormone (FSH, Follitrophin)
Force Fields/Molecular Mechanics
Forskolin
FoxO1a
Frizzled
Frontal Cortex
FSH
Functional Genomics
Functional Magnetic Resonance Imaging (fMRI)
Fungi
Fungicidal Effect
Fungistasis
Funicular Myelitis
Furin
Furin-like Protease
Fyn
FYVE Domain
Fz Receptors
G-actin
G-Proteins
GABA
GABA_A Receptors
GABA_B Receptors
[GABAergic System](#)
Gaddum
Galanin
[Galanin Receptors](#)
Galanin-like Peptide (GALP)
GAPs
Gasotransmitters
Gastric H,K-ATPase
Gastric Inhibitory Peptide (GIP)
Gastrin
Gastrin Releasing Peptide
Gastroesophageal Reflux Disease (GERD)
Gating
G-CSF
GDIs
GEFs
Gene Activity Profile
Gene Array
Gene Chip
[Gene Expression Analysis](#)
Gene Gun
Gene Products
Gene Promoter
Gene Therapy
[Gene-therapy Vectors](#)
Gene Transfer
Gene Transfer Mechanisms (Bacteria)
[General Anaesthetics](#)
General Transcription Factors
Genetic Polymorphism
Genome-wide Association Study (GWA Study)
Genomics
Genotype

Gephyrin
 GERD
 GH
 Ghrelin
 Giardia lamblia
 GIP
 GIRK
 Gitelman's Syndrome
 Glial Cells
 Glibenclamide
 Glomerular Filtration Rate
 GLP-1
 Glucagon
 Glucagon-like Peptide-1 (GLP-1)
 Glucocorticoid Receptor
 Glucocorticoid Resistance
[Glucocorticoids](#)
 Glucocorticoids, Inhalable
 Glucocorticosteroids
[Glucoc-mineralocorticoid Receptors](#)
 Gluconeogenesis
 Glucose Transport Facilitators (GLUT)
[Glucose Transporters](#)
 Glucose/Galactose Malabsorption (GGM)
 α -Glucosidase
 GLUT
 GLUT2
 GLUT4
 Glutamate
 Glutamate Receptors
 γ -Glutamyl-transpeptidase
 Glycine
[Glycine Receptors](#)
 Glycogen Synthase Kinase 3
 Glycopeptide Antibiotics
 Glycoprotein IIb/IIIa Receptor Antagonists
 Glycoproteins
 Glycosaminoglycan
 Glycosides, Cardiac
 Glycosylation
 Glycosylphosphatidylinositol Anchor (GPI Anchor)
 Glycylcyclines
 Glypiation
 GM-CSF
 GM2-gangliosidosis
 GMP, Cyclic
 GnRH
 Gold Compounds
 Golgi Apparatus
 Gonadorelin
 Gonadotropin-releasing Factor/Hormone (GnRH)
 Gonadotropins
 Gout
 GPCRs
 GPI Anchor
 GPR30
 G-protein-coupled Receptor Kinases (GRKs)
[G-protein-coupled Receptors](#)
 G-proteins and Wnt Signaling
 Granulocyte-CSF (G-CSF)
 Granulocyte-macrophage-CSF (GM-CSF)
 Graves' Disease
 Grb-2
 GRKs
 Growth
[Growth Factors](#)
 Growth Hormone
 Growth Hormone Release-inhibiting Factor
 GSK3
 GTPase Activating Proteins (GAPs)
 GTPases
 Guanine Nucleotide Dissociation Inhibitors (GDIs)
 Guanine Nucleotide Exchange Factors (GEFs)
[Guanylyl Cyclase](#)
 Gyrase
 Haemostasis
 Half-life/Elimination Half-life ($t_{1/2}$ or $t_{1/2\beta}$)
 Hamartoma Syndromes
 Haplotype
 Hashish
 HCN
 HCV
 HDL
 Heat Shock Protein (HSP)
 Helicases
 Helicobacter Pylori
 Helix Bundle
 Helix-loop-helix Motif
 Helper T Cells
 Hemangioblast
 Hematopoiesis
[Hematopoietic Growth Factors](#)
 Heme
 Hemodialysis
 Hemofiltration
 Hemostasis
 Hemozoin
 Heparin Sulfate Proteoglycans
 Hepatic Lipase
 Hepatitis
 Hepatitis C
 Heptahelical Domain
 Heptahelical Receptors
 HERG-channels
 Heterologous Desensitization
 Heterologous Expression System
 Heterotrimeric G-Proteins
 Hidden Markov Model
 High-density Lipoprotein (HDL)
[High-throughput Screening](#)
 High-voltage-activated Ca^{2+} Channels
 Hill Analysis

Hippocampus
Hirudin
Histamine
Histamine and Emesis
[Histaminergic System](#)
Histone
Histone Acetyl Transferases (HAT)
[Histone Acetylation](#)
Histone Deacetylases
Histone Methylation
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Histone Tails
HIV
HMG Box
HMG CoA-Reductase
[HMG-CoA-Reductase Inhibitors](#)
Homologous Desensitization
Homologous Proteins
Homologous Recombination
Homology Modeling
Hormonal Contraceptives
Hormone Replacement Therapy (HRT)
HPA Axis
HRT
Hsp
Hsp70
HSV
5-HT
Human Leucocyte Antigens (HLA)
[Humanized Monoclonal Antibodies](#)
Humoral Immunity
HVA Ca^{2+} Channels
Hybridization
6-Hydroxydopamine
Hydroxypyridones
11 β -Hydroxysteroid Dehydrogenase Type II
5-Hydroxytryptamine (5HT)
Hyoscine (Scopolamine)
Hyperactivity Disorder
Hyperaldosteronism
Hyperalgesia
Hypercholesterolemia
Hyperekplexia
Hyperglycaemia
Hyperinsulinemic Hypoglycemia of Infancy (HI)
Hyperkalemia
Hyperlipidaemia/Hyperlipoproteinaemia
Hyperplasia
Hyperpolarization-activated and Cyclic Nucleotide-gated Channels (HCN)
Hyperpyrexia
Hypersensitivity
Hypertension
Hyperthyroidism
Hyperuricemia
Hypnotic
Hypoaldosteronism
Hypocretins
Hypoglycaemia
Hypogonadotropic Hypogonadism
Hypokalemia
Hypomethylating Agents
Hyposensitization
Hypotension
Hypothalamus
Hypothalamus–Pituitary–Adrenal (HPA) Axis
Hypothyroidism
Hypoxia
IC₅₀ Values
Idiopathic Hypercalciuria
Idiosyncratic Reactions
IGF
IKK Complex (IKK Family)
IKr
Imidazoline Receptor
Immediate Early Genes
Immune Complexes
[Immune Defense](#)
Immune System
Immunity
Immunoglobulins (Immunoglobulin Superfamily)
Immunomodulators
Immunophilins
[Immunosuppressive Agents](#)
IMPDH
Importins
In Silico Biology
Inclusion Cell Disease
[Incretin Hormones](#)
Inducible (Immunological) Nitric Oxide Synthase (iNOS)
[Inflammation](#)
Inflammatory Caspases
Inflammatory Disorders
Influenza
Infrared Spectroscopy
Innate Immunity
Inorganic Phosphate Transporters
iNOS
Inositol
Inositol 1,4,5-trisphosphate Receptors
Insomnia
Insulin
Insulin Desensitization
[Insulin Receptor](#)
Insulin Resistance
Insulin Secretagogues
Insulin-like Growth Factor
Integrase
Integrin, α 4 β 1
Integrin, α 4 β 7
Integrin, α IIb β 3

Integrin, α v β 3
 Integrin, α v β 5
 Integrins
 Integron
 Interactions
 Interfacial Inhibitors
 Interferon Stimulated Gene 15
[Interferons](#)
 Interleukin-1 (IL-1)
 Interleukin-2 (IL-2)
 Interleukin-4 (IL-4)
 Interleukin-5 (IL-5)
 Interleukins
 Intermediate Filaments
 Intermediate-density Lipoprotein (IDL)
 Intermittent Claudication
 Internalization
 International Normalized Ratio (INR)
 Intima
[Intracellular Transport](#)
 Intrathecal Application
 Intrathecal Space
 Intrinsic Efficacy
 Intron
 Inverse Agonist
 Inward Rectification
[Inward Rectifier K⁺ Channels](#)
 Iodide
 Iodine
 Ion Channels
 Ionic Contrast Media
[Ionotropic Glutamate Receptors](#)
 Ionotropic Receptors (Channel-linked)
[IP₃ Receptors](#)
 IPC
 IPSP (Inhibitory Postsynaptic Potential)
 IRAG
 IRAK Family
 Iron Chelator
 Irreversible Antagonists
 Irritable Bowel Syndrome (IBS)
 Isaacs' Syndrome
 Ischemic Preconditioning (IPC)
 Ischemic Stroke
 Isopeptide Bond
 Isoprenoid
 Isoprenylation
 ITAM
 Itch
[JAK-STAT Pathway](#)
 JNKs
 K_{ATP} Channel
 K_{NDP} Channel
 K⁺ Channel Openers
 K Channels
 K_D
 K⁺ Homeostasis
 k_{off}
 Kainate Receptor
 Kallidin, Lysyl-Bradykinin
 Kallikrein
 KCNQ-Channels
 KCOs
 KDEL Receptor
 KELL Blood Group Antigen
 Ki Values
 Kinase
 Kinase Domain
 Kinase Inhibitors
[Kinins](#)
 Kir Channels
 Knockout Mice
 Kringle Domains
 K⁺-Sparing Diuretics
 Kv β -Subunits
 Kv-Channels
 KvLQT1-Channels
 Kynurenine Pathway
 L-NAME
 [\$\beta\$ -Lactam Antibiotics](#)
 β -Lactamases
 Lateral Hypothalamic Area
 Laxatives
 LCAT
 LD₅₀
 LDL-Cholesterol
 LDL-Receptors
 Lead
 Lead Discovery by NMR
 Lecithin-Cholesterol Acyltransferase
 Leonardo
 Leptin
 Lethal Dose
 Leucin Zipper
[Leukotrienes](#)
 Levodopa
 Lewy Bodies
 LH-RH Agonist
 Liddle's Syndrome
 Ligand
 Ligand-Gated Ion Channels
 Limbic System
 Linkage Disequilibrium (LD)
 Lipid-lowering Drugs
 Lipid Metabolism
[Lipid Modifications](#)
 Lipid Phosphate Phosphohydrolases
 Lipid Rafts
[Lipid Transfer Proteins](#)
 Lipidation
 Lipopolysaccharide
 Lipoprotein Lipase

[Lipoprotein Metabolism](#)

Lipoproteins

Liposomes

5-Lipoxygenase

Lipoxygenases

Lithocholic Acid

[Local Anaesthetics](#)

Locus

Locus Coeruleus

Long-Chain Fatty Acids

Long-QT Syndromes

Long-Term Depression

Long-Term Potentiation

Loop Diuretics

Low-density-lipoprotein-cholesterol

[Low-density Lipoprotein Receptor Gene Family](#)

Low-density Lipoprotein Receptor-related Proteins

Low Molecular Mass GTPases

Low Voltage-activated (LVA) Ca^{2+} Channels

LPL

LPS

LRP

LRP5 and LRP6

LTD

LTP

Luteinizing Hormone

Lymphangiogenesis

Lymphocytes

Lymphokines

Lysine Acetylation of Histones

Lysipressin

Lysolipid Mediators

Lysophosphatidic Acid

Lysophosphatidylcholine

[Lysophospholipids](#)

Lysosphingolipids and Lysoglycerophospholipids

M-Channels

Macrolides

Macrophage

Macula Densa

Major Histocompatibility Complex (MHC)

Malaria

Malignant Hyperthermia (MH)

Malignant Neoplasm

Malignant Tumor

Mannose 6-Phosphate Receptor

MAO

MAP

[MAP Kinase Cascades](#)

MAPK

Marijuana

Marimastat

Mast Cells

[Matrix Metalloproteinases](#)

Matrix-assisted Laser Desorption/Ionization Mass Spectrometry

Maturity-Onset Diabetes of the Young (MODY)

MCH

MCR

MDMA

[MDR-ABC Transporters](#)

Mechanism-based Inhibition

Meglitinide-related Compounds

Melanin-concentrating Hormone

[Melanocortins](#)

Melanocyte-stimulating Hormone

Melatonin

Membrane Signal Transduction

Membrane Transport Protein

Menaquinones-n

Ménière's Disease

Mesangial Cells

Mesolimbic System/Reward System

Metabolic Syndrome

[Metabotropic Glutamate Receptors](#)

Metabotropic Receptor

Metalloprote(in)ases

Methicillin-resistant Staphylococci

N-Methyl D-aspartate Receptors

Methylating Agents

3,4-Methylenedioxymeth-Amphetamine

2-Methyl-6-(phenylethynyl) Pyridine

1-Methyl-4-Phenylpyridium

1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine

Methylxanthines

MHC

MIC

Michaelis–Menten Kinetics

Microarray

Microarray Hybridization

[Microarray Technology](#)[Microbial Resistance to Drugs](#)

Microfilaments

Microtubule

Microtubule Associated Proteins

Mineralcorticoids

Mineralocorticoid Receptor (MR)

Minimal Inhibitory Concentration

MinK

MirP Subunits

Mitochondrial Permeability Transition Pore

Mitogen

Mitogen-activated Protein Kinase

Mitosis

Mixed Agonists/Antagonists

Mixed Function Oxygenase

MLCK

MLCP

MMPs

Molecular Cancer Therapeutics

Molecular Dynamics

Molecular Dynamics Simulation

[Molecular Modeling](#)

Molecular Simulation

Monoamine Oxidases

[Monoamine Oxidases and their Inhibitors](#)

Monoclonal Antibody

Monocytes

Monokines

Monophasic Preparations

Monoxide

Mood Disorders

Mood Elevators

Mood-stabilising Drugs

Morbus Alzheimer

Morphogens

Morpholines

Motilin

Motion Sickness

Motor Proteins

MPEP

MPP⁺

MPTP

mRNA

MRP

MS/MS

mTOR, replaces FRAP, RAFT, RAPT or SEP in mammals

Mucolipin

Multidrug Resistance

Multidrug Resistance Gene

Multiple Sclerosis

Muscarinic Acetylcholine Receptors

[Muscarinic Receptors](#)

Muscle Relaxants

Muscle Type Nicotinic Receptors

Myasthenia Gravis

Myelin Basic Protein

Myelosuppression

Myocardial Infarction

Myosin Light Chain Kinase

Myosin Phosphatase (MLCP)

Myotonia

Myristoylation

N-Myristoylation

Na⁺/Ca²⁺ AntiporterNa⁺/Ca²⁺ Exchanger[Na⁺/Ca²⁺ Exchangers](#)Na⁺ Channel

NaCl Cotransporter

Na⁺-dependent Glucose CotransporterNa⁺-dependent Glucose Cotransporter[Na⁺/H⁺ Exchangers](#)Na⁺- and K⁺ activated Adenosine 5'-Triphosphatase[Na⁺/K⁺-ATPase](#)Na⁺ K⁺ 2Cl⁻ CotransporterNa⁺-Proton Exchanger

NADPH-diaphorase

NANC Transmission/Mediators

Narcolepsy

Narcotics

Natriuretic Drugs

Natriuretic Peptides

Natural Killer Cells

Nausea

NCX

NDP-dependent K⁺ Channel

Necrosis

NEP

Nephrogenic Diabetes Insipidus

Neprilysin

Nerve Growth Factor

Neuraminidase Inhibitors

Neuraxis

Neuregulin

[Neurodegeneration](#)

Neurokinin A

Neuroleptic-like Malignant Syndrome

Neuroleptics

Neuromedin B

Neuromedin N

Neuromedin U

Neuromodulators

Neuromuscular Blocking Agents

Neuromuscular Junction

Neuronal Cell Death

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Neurotrophin

Neutral Antagonist

Neutral Endopeptidase

Neutropenia

Neutrophils

NF-κB

NF-IL6

[NFAT Family of Transcription Factors](#)

NGF

NHE

[Niacin](#)

Nicotinic Acetylcholine Receptor

[Nicotinic Receptors](#)

Nigrostriatal Tract/Pathway

Nitrates

Nitrgic Nerve
Nitrgic Transmission
[Nitric Oxide](#)
Nitric Oxide Synthase (NOS)
N-nitro-L-arginine Methyl Ester
Nitrogen Mustards
Nitrosourea Derivatives
Nitrovasodilators
NK Cells
NK Receptors (Neurokinins Receptors)
NMDA
NMDA Receptor
NMR
NMR-Screening
NO
NO Donator
[NO Synthases](#)
Nociceptin/Orphanin FQ
Nociception
Nociceptive Transducers
Nociceptors
Nodes of Ranvier
Non-adrenergic Non-cholinergic (NANC)
 Transmission/Mediators
Non-competitive Antagonism
Non-competitive Inhibition
Nonionic Contrast Media
Non-neuronal Monoamine Transporters
Non-opioid Analgesics
Non-rapid Eye Movement (NREM) Sleep
Nonreceptor Tyrosine Kinases
[Non-selective Cation Channels](#)
Non-selective Monoamine Reuptake Inhibitor
Non-Specific ChE
[Non-steroidal Anti-inflammatory Drugs](#)
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Noradrenaline Transporter
Norepinephrine/Noradrenalin
Normoglycaemia
NOS
NPA Motif
NPY
NPY₃₋₃₆
NSAIDs
NSAR
NSF
NSMRI
Ntn-Hydrolase
[Nuclear Factor Kappa B](#)
Nuclear Factor of Activated T Cells Cytosolic NFATc
Nuclear Localization Signal
Nuclear Pore Complex
[Nuclear Receptor Regulation of Hepatic Cytochrome
 P450 Enzymes](#)
[Nuclear Receptors](#)
Nucleic Acid Vaccination
Nucleophilic
Nucleosome
Nucleus Accumbens
Obesity
OCT
Oedema
Oestrogens
Off-resin Analysis
Olfactory Epithelium
Olfactory Receptors
Oligonucleotide
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On-resin Analysis
Oncogenes
Oncogenesis
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Opiates
Opioid Receptors
 σ -Opioid Receptors
[Opioid Systems](#)
Opium
Orai Proteins
Oral Anticoagulants
Oral Blood Glucose Lowering Drugs
Oral Contraceptives
Oral Hypoglycaemic Agents
Orexigenic
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Organic Cations
Organic Nitrates
Organophosphates
ORL-1
Orphan G-protein-coupled Receptors
Orphan Nuclear Receptors
[Orphan Receptors](#)
Orphan Serpentine Receptors
OrphaninFQ/Nociceptin
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Osteoarthritis
Osteoblast
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Osteocyte
Osteoporosis
Osteotropic Hormones
Ototoxicity
Oxazolidinones
Oxidase, Mixed Function
Oxidative Stress
Oxytocin
P1 Receptors
p53
p70S6 Kinase
P450 Induction

[P450 Mono-oxygenase System](#)
 P-type ATPase(s)
 PACAP
 PAF
 PAG
[Pain and Nociception](#)
 Pain Medication
 Palmitoylation
 PAMPs
 Pancreatic β -cell
 Pancreatic Polypeptide
[Pantothenic Acid](#)
 Parasite
 Parasympathetic Nervous System
 Parasympatholytics
 Parasympathomimetics
 Parathyroid Hormone
 Paraventricular Nucleus
 Parkin
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 Partial Agonists
 Parvalbumin
 PAS Domain
 Passive Diffusion
 Patch-Clamp Method
 PC: Proconvertase
 PCI
 PDE
 PDGF
 PDZ Domain
 PEG
 Pellagra
 Pemphigus Blistering Disease
 Penicillin
 Penicillin Binding Protein
 Pentasaccharide
 Peptide Mass Fingerprint
 Peptide YY
 Peptidoglycans
 Peptidyl Transferase Center
 Peptidyl-Dipeptidase
 PER1
 Percutaneous Coronary Intervention
 Periaqueductal Grey
 Pericytes
 Peripheral Nerve
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[Peroxisome Proliferator-Activated Receptors](#)
 Peroxynitrite
 Pertussis Toxin
 PEST Sequences
 PGI₂
 P-Glycoprotein
 PH Domain
 PH-Sensing Receptors
 Phage
 Phagocyte/Phagocytosis
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[Pharmacogenetics](#)
[Pharmacogenomics](#)
[Pharmacokinetics](#)
 Pharmacological Barriers
 Pharmacological Chaperone
 Pharmacophore Modelling
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 Phase I and Phase II Metabolism
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 Phenothiazines
 Phenotype
 Phenylalkylamines
 PHHI
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 Phorbol Esters
 Phosphatase
 Phosphate Transferring Enzyme Inhibitors
 Phosphatidic Acid
 Phosphatidylinositol Kinases
 Phosphatidylinositol
 Phosphatidylinositol 4,5-bisphosphate
 Phosphatidylinositol 3-kinase
 Phosphatidylinositol Kinases
 Phosphatidylinositol Phosphate
 Phosphatidylinositol
[Phosphodiesterases](#)
 Phosphoinositol Kinases
[Phospholipases](#)
 Phospholipid
[Phospholipid Kinases](#)
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 Phthalate Esters
 Phylloquinones
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 PI Response
 PIAS
[PIAS Proteins](#)
 PIC1 (PML Interacting Protein 1)
 Picornaviruses
 Picrotoxin
 PI3-Kinase
 PIP
 PIP₂
 Pituitary Adenyl Cyclase-Activating Polypeptide
 PKA
 PKB/Akt
 PKC
 PKD2
[Placebo Effect](#)
 Placebo Response
 Placenta Growth Factor

Planar Cell Polarity Pathway
Plaque
Plasma Binding
Plasma ChE
Plasma Lipid Transfer Proteins
Plasmid
 α_2 -Plasmin Inhibitor
Plasmin(ogen)
Platelet-activating Factor
Platelet Aggregation Inhibitors
Platelet-derived Growth Factor Receptor
Platelet Inhibitors
Platelets
Platinum Complexes
Pleckstrin Homology Domain
[Plexins](#)
PMF
Polyamines
Polyenes
Polyethylene Glycol
Polymorphism
Polypeptide Chain Binding Proteins
POMC
Poor Metabolizer Phenotype
Population Pharmacokinetics
Positron Emission Tomography
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[Potassium Channels](#)
Potassium Competitive Acid Blockers
PP
PPAR
PPCs
PR
Pregnane X Receptor
Preintegration Complex
Prenylation
Prepro-opiomelanocortin
Primary Hemostasis
Primary Hyperparathyroidism
Prodrug
Progesterone Receptor
Progestins
Programmed Cell Death
Prolactin
Proopiomelanocortin
Propionylcholinesterase
Prostacyclin
Prostaglandin H2 Synthase
Prostaglandins
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Protamine Sulfate
Protease-activated Receptors
Proteases
Proteasome
Protein Degradation
Protein Folding

Protein Folding Problem
Protein Kinase
Protein Kinase A
[Protein Kinase C](#)
[Protein Kinase Inhibitors](#)
[Protein Phosphatases](#)
Protein Sorting
[Protein Trafficking and Quality Control](#)
Protein Transport
Protein Tyrosine Kinases
[Proteinase-activated Receptors](#)
Proteinases
[14-3-3 Proteins](#)
Proteolytic Enzymes
Proteome
[Proteomics](#)
Proteosomal Degradation
Prothrombin Time
Protocadherins
Protocidal Drugs
[Proton Pump Inhibitors and Acid Pump Antagonists](#)
[Proton-Sensing GPCRs](#)
Proto-oncogene
PRRs
PSD-95
Pseudocholinesterase
Pseudogene
P-Site Ligands
Psoralens
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Psycho Energizers
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[Psychostimulants](#)
Psychotogenic
[Psychotomimetic Drugs](#)
PT
PTB Domain
PTEN
PTM
Pulmonary Emphysema
Pulmonary Hypertension
[Purinergic System](#)
Purinoceptor
PXR
Pyknosis
Pyramidal Cell
Pyrexia
Pyrogenic Cytokines
PYY₃₋₃₆
QA/QC Control Metrics
QT Prolongation
Quantification of Drug Effect
Quantitative PCR
Quinolinic Acid
[Quinolones](#)

RAAS
 Rab-GTPases
 Radiocontrast Agents
 Radioiodine
 Radiomimetics
 Raf
 RAGE
 Rapamycin Analogs
 Raphe Nuclei
 Rapid Eye Movement Sleep
 Ras Protein
 Raynaud's Phenomenon
 Rb Gene
 Reactive Oxygen Species
 Rebound Insomnia
 Receptor for Advanced Glycation End Products
 Receptor Occupancy
 Receptor Protein
 Receptor Reserve
 Receptor Serine/Threonine Kinases
 Receptor Subtype
 Receptor Tyrosine Kinases
 Receptors
 Recombinant Protein
 Rectification
 5 α -Reductase
 Reelin
 Reentrant Arrhythmia
 Regulated Secretion
 Regulation of Gene Expression
 Regulation of Ingestive Behaviour
 Regulation of mRNA Synthesis
 Regulation of Smooth Muscle Contractility
 Regulators of G-protein Signalling
 Regulatory Myosin Light Chain II
 Regulatory Regions
 Regulatory T-cells
 Reinstatement
 Rel Homology Domain
 Rel/NF- κ B
 Rel Proteins
 Relative Potency
 Relaxin
 REM Sleep
 Renal Glucosuria
[Renin–Angiotensin–Aldosterone System](#)
 Repolarization
 Resiniferatoxin
 Resistance Plasmid
 Respiratory Burst
 Restenosis
 Resting Potential
 Resting Tremor
 Restless Leg Syndrome
 Retinal
 Retinitis Pigmentosa
 Retinoblastoma (Rb) Gene
 Retinoic Acid
 Retinoic Acid Receptors
 Retinoid-resistance
 Retinoid X Receptor
[Retinoids](#)
 Retinol
 Retinyl Esters
 Retrograde Messenger
 Retroviruses
 Reuptake Transporter
 Reverse Complementary Oligonucleotides
 Reverse Mode
 Reverse Pharmacology
 Reverse Transcription
 Reward (Pathways)
 Reye's Syndrome
 RGS Protein
 Rhabdomyolysis
[Rheumatoid Arthritis](#)
 Rheumatoid Factor
 Rho
 Rho-kinase
 Riboflavin
[Ribosomal Protein Synthesis Inhibitors](#)
 Ribosome-targeting Antibiotics
 Ribozymes
 Rigidity
 RING
 RNA Editing
[RNA Interference \(RNAi\) – siRNA](#)
 RNA Polymerase
 RNA Polymerase III Promoter
 RNA Polymerase Inhibitors
 RNAi
 RNases
 RTK
 RT-PCR
 RXR
[Ryanodine Receptor](#)
 Ryk/Derailed
 S6K1
 S-Prenylation
[S100 Proteins](#)
 S-Acylation
 S-adenosyl-L-methionine
 Salicylate
 SAM
 SAR
 SARA
 Sarafotoxin
[SAR-by-NMR](#)
 Sarcolemma
 Sarcoplasmic Reticulum
 SARMs
 Saxitoxin

Scaffold
Scaffolding Proteins
Scatchard Plot
Scavenger Receptors
Schild Analysis
Schizophrenia
Second Messenger
Secretase
Secretory Pathway
Sedatives
Seizure
Selectins
Selective Androgen Receptor Modulator
Selective Estrogen Receptor Downregulator
Selective Estrogen Receptor Modulators
Selective Noradrenaline Reuptake Inhibitors
Selective Progesterone Receptors Modulators
Selective Serotonin Reuptake Inhibitors
[Selective Sex Steroid Receptor Modulators](#)
Self Tolerance
Sema Domain
Semaphorins
Senescence
Sensitization
Sensory Nerves
Sentrin
Septicemia
Sequence Contig
Sequence Profile
Sequence-specific Transcription Factors
Sequential Preparations
SERCA
Serine-hydroxymethyltransferase
Serine Proteinases
Serine/Threonine Kinase
SERM
Serotonin
[Serotonergic System](#)
Serpentine Receptors
Serum Sickness
Seven Transmembrane Helix Receptors
[Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor](#)
SGLT
SH2, Domains
SH3 Domain
Shaker-Channels
Shine-Dalgarno Interaction
SIADH
Sialic Acid
Sialin
Signal Peptidases
Signal Recognition Particle
Signal Transducer and Activator of Transcription
Simple Diffusion
Simulated Annealing
Single Nucleotide Polymorphism
Sinus Rhythm
siRNAs
Skeletal Metabolism/Turnover
Skinned Fiber
Skp1/Cul1/F-Box Protein Complex
[Sleep](#)
Sleep Apnea
Sleep Continuity
Sleep (Onset) Latency
Sleep Quality
Sleep Stages
Sleeping Sickness (African)
Slow Wave Activity
Slow Wave Sleep
Smad
Small G Proteins
[Small GTPases](#)
[Smooth Muscle Tone Regulation](#)
SMURF
SNAPs
SNARE Proteins
SNF1 Complex Au2 (Fungi)
SNPs
SNRIs
SOCS
SOD
Solid Phase Synthesis
Soluble Guanylyl Cyclase
Solute Carrier
Somatomedins
[Somatostatin](#)
Somatotropin Release Inhibitory Factor
SOS
Spermidine
Spermine
Sphingosine Kinases
Sphingosine-1-phosphate
Sphingosylphosphorylcholine
Spike-ins
Spindle Assembly Checkpoint
Splice Variant
Splicing
Sporotrichosis
SPRM
SR
Src Gene
Src-homology 2 Domain
Src-homology 3 Domain
Src Kinase
SRP
SSRIs
SST14
SST28
STAT
State-dependent Block

Statins
 Status Asthmaticus
 Steroid Receptors
 Steroids
[Sterol Transporters](#)
 STIM
 Stimulant Drugs
 Store-operated Ca^{2+} Entry
 Streptokinase
 Stress
 Stress Proteins
 Structural Bioinformatics
 Structural Genomics
 Strychnine
 STX
 Substance K
 Substance P
 Substance Use Disorders
 Substantia Gelatinosa
 Substantia Nigra
 Sulfonylurea Receptor
 Sulfonylureas
 Sulfotransferase
 SUMO
[SUMOylation](#)
 Superantigen
 Supercoils
 Superfamily
 Superoxide
 Superoxide Dismutase
 Suprachiasmatic Nucleus
 Supraventricular Tachycardia
 SUR
 Surrogate Ligand
 Susceptibility
 Swiss-Prot
 SxxK Acyl Transferases
 SxxK Free-standing Penicillin-binding Protein
 SxxK β -lactamases
 SxxK PBP Fusions
 Sympatholytic Drugs
 Sympathomimetic Drugs
 Synapse
 Synaptic Plasticity
[Synaptic Transmission](#)
 Synaptic Vesicle Neurotransmitter Transporters
 Synaptic Vesicles
 Synaptotagmin
 Syndecans
 Synergistic Interaction
 Systems Biology
 Systolic and Diastolic Blood Pressure and Pulse Pressure
 t1/2 or t1/2 β
 T3
 T4
 T Cell Anergy
 T Cell Antigen Receptor
[T Cell Receptors](#)
 T-cells
[Tachykinins and their Receptors](#)
 Tachyphylaxis
 Tacrolimus
 Tandem Mass Spectrometry
 Tardive Dyskinesia
 Target of Rapamycin Encoded by: TOR1 and TOR2 in Yeast
[Targeted Cancer Therapy](#)
 Taste Receptors
 TATA Box
 Taxanes
 TCR
 TDM
 Temporal Lobe
 Tendinitis
 Teratogen
 Testosterone
 Tetanus
 Tethered Ligand
 Tetracycline
 Δ^9 -Tetrahydrocannabinol (THC)
 3',5',3,5-L-Tetraiodothyronine
 Tetramer
 Tetradotoxin
 TF
 Tg
 TGF- β Receptor Signaling Pathway
 TH
 Thalamocortical Synchronisation
 THC
 Therapeutic Drug Monitoring
 Thiamin
 Thiazide Diuretics
 Thiazolidinedione
 Thienopyridines
 Thionamides
 Thiopurine S-methyltransferase
 Thioxanthene
 Threading
 Thrifty Gene Hypothesis
 Thrombin
 Thrombin Receptors
 Thrombocytopenia
 Thrombolysis
 Thrombopoietin
 Thrombosis
 Thromboxane
 Thrombus
 Thymoleptics
 Thymus
 Thyroglobuline
 Thyroid Autonomy

Thyroid Peroxidase
Thyrotoxicosis
Thyroxine
TIMP
TIR Domain
Tissue Factor
Tissue-specific Agonists/Antagonists
Tissue-type Plasminogen Activator
T-kinin (Ile-Ser-Bradykinin)
TKIs
TLR
T-lymphocytes
TNF
TNF Receptor Associated Factors
TNF Receptor Superfamily
Tocolytics
Tolerance
[Tolerance and Desensitization](#)
[Toll-like Receptors](#)
Tonic-clonic Convulsions
Topoisomerase
Topotecan
[TOR Signalling](#)
Torsade de Pointes
Total Sleep Time
Toxicogenomics
[Trace Amines](#)
TRAF
Trafficking
Tranquilizers
Transactivation
Transcription Factor
Transcriptional Coactivators
[Transcriptional Regulation](#)
Transcriptome
Transcriptome Analysis
Transduction
Transfection
[Transforming Growth Factor-Beta](#)
Transforming Oncogenes
Transgene
[Transgenic Animal Models](#)
Transient Receptor Potential
Transition State
Translation Inhibitors
Translocon
[Transmembrane Signaling](#)
Transport ATPase
Transporter
Transposon
Transverse Tubule
Triazenes
Trichomonas Vaginalis
Tricyclic Antidepressants
Triiodothyronine
3',5,3-L-Triiodothyronine
Trimodal Distribution
Tripeptidyl-peptidase
Tropism
Troponin
[TRP Channels](#)
TRPM4
TRPM7
TRPM8
TRPV1
TRPV4
TRPV5 and TRPV6
True ChE
Trypsin
Trypsin-like Proteinases
TSH Receptor
T-tubule
TTX
Tubulin
 α and β Tubulin
[Tumor Necrosis Factor](#)
Tumor Suppressor Genes
TUNEL
Turing Machine
Twitch/Tetanus
Two-dimensional Gel Electrophoresis
Two Metal Sites
L-Type Ca^{2+} Channel
Type I Allergic Reaction
Type I Transmembrane Proteins
Type II Allergic Reaction
Type II Transmembrane Proteins
Type III Allergic Reaction
Type IV Allergic Reaction
Typical Antipsychotic Drugs: Neuroleptic Drugs
Tyrosine Hydroxylase
[Tyrosine Kinase Inhibitors](#)
[Tyrosine Kinases](#)
Tyrosine Phosphatases
Tyrosylprotein Kinase
Tyrphostins
Ubiquinone
Ubiquitin-Dependent Protein Degradation
[Ubiquitin/Proteasome](#)
Ubl1
UDP-Glucuronyl Transferase
Ultrarapid Metabolizer
Unstable Angina
Unwanted Effects
UPR
Uptake Transporter
Urate
Urea Transporter
Uric Acid
Uricosstatic Drug
Uricosuric Drug
Urodilatin

Urographic Contrast Agents
Urokinase-Type Plasminogen Activator
Urotensin
Urticaria
UT1
V-type ATPase
Vaccination
VACHT
Vacuolar-type Proton Translocating ATPase
Vanilloid Receptor
Varizella Zoster Virus
[Vascular Endothelial Growth Factor](#)
Vasoactive Intestinal Peptide
Vasoconstrictor
Vasodilator
Vasopeptidase Inhibitors
[Vasopressin/Oxytocin](#)
Vasorelaxant
Vav
VEGF
Venus Flytrap Module
Very Long-chain Acyl-CoA Synthetase
Very Low-density Lipoprotein
Vesicle
Vesicular Acetylcholine Transporter
[Vesicular Transporters](#)
VFT Domain
VGAT/VIAAT
VGLUT
Vinca Alkaloids
VIP
VIPomas
[Viral Proteases](#)
Viral Vectors
Virostatics
Virus-like Particle
Vitamin A
[Vitamin B1](#)
[Vitamin B2](#)
[Vitamin B6](#)
[Vitamin B12](#)
[Vitamin C](#)
Vitamin D
[Vitamin E](#)
[Vitamin K](#)
VLCS
VLDL
VMAT
[Voltage-dependent Ca²⁺ Channels](#)
Voltage-dependent Inactivation
[Voltage-dependent Na⁺ Channels](#)
[Voltage-gated K⁺ Channels](#)
Voltage-gated Na⁺ Channel
Voltage Sensor
Vomiting, Act of
von Willebrand Factor
vWf
VZV
Wall Peptidoglycan Inhibitors
Water Channel
Watson-Crick Base Pairing
Weight-loss Therapies
Wernicke's Syndrome/Encephalopathy
Wheal
Wheezing
Wingless
Withdrawal
[Wnt Signaling](#)
Wnts and Vertebrate Development
Wortmannin
WW Domain
Xanthine Oxidase
Xanthines
Xenobiotic
X-linked Nephrogenic Diabetes Insipidus
X-ray Contrast Agents
[X-ray Contrast Agents and Molecular Imaging](#)
Y-27632
YAC
Zellweger Syndrome
Zero-Order Kinetic
ZES
Zinc Finger
Zollinger-Ellison Syndrome
Zona Glomerulosa
Zymogen